VOL. XIV. [DECEMBER, 1892.] No. 8.

AMERICAN

CHEMICAL JOURNAL.

Contribution from the Chemical Laboratory of Clark University, Worcester, Mass.

THE ADDITION-PRODUCTS OF BENZO- AND OF TOLUQUINONE.

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The presence of two pairs of doubly-bound carbon atoms in benzoquinone has been made highly probable by the work of Nef,[2] who obtained a dibromide and a tetrabromide. The following experiments, which have been carried out under the direction of Dr. Nef, are a continuation of the study of the addition-products formed by quinones. Up to the present time five addition-products of quinone have been obtained, namely a di- and a tetrabromide, a di- and a tetrachloride and a dichloride-dibromide. These five compounds are all strictly analogous and quite stable —the chlorine derivatives most so.

The dichloride and dibromide have a faint quinone-like odor, are light-colored, moderately soluble in organic solvents, and soluble without change in fuming nitric acid. The other three are colorless, inodorous, and very sparingly soluble in organic solvents; they dissolve with difficulty and without change in warm fuming nitric acid. All five products are instantly decomposed by dilute sodic hydroxide; they all split off halogen hydride (one

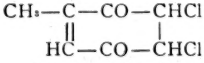
[1] Accepted as a thesis for the degree of Doctor of Philosophy at Clark University, June, 1892.

[2] This Journal **12**, 483; **13**, 423.

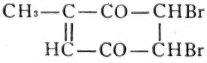
or two molecules) on warming the alcoholic solutions, and go over into one, or two isomeric, halogen-substituted benzoquinones. Treated with zinc and acetic acid, all the halogen atoms are eliminated and hydroquinone is obtained from all.

The decomposition-products of quinone dichloride-dibromide formed by boiling it in alcoholic solution are meta- and parachlorbromquinone; and this proves, beyond a doubt, that the addition of halogen, as well as the splitting off of halogen hydride from the resulting addition-products, takes place exclusively in the ortho position, as has already been elucidated in a previous paper.[1]

Toluquinone forms a dibromide and a dichloride, but all attempts to obtain a tetra-halogen addition-product were fruitless. The products obtained closely resemble the corresponding benzoquinone bodies. Reduced with zinc and acetic acid, the halogen atoms are taken out and hydrotoluquinone results. On boiling an alcoholic solution of toluquinone dichloride it loses one molecule of hydrochloric acid, and meta- and parachlortoluquinone are formed. This proves that the addition of the halogen atoms has taken place, not on the propylene side of the toluquinone molecule, as was expected, but on the ethylene side; *i.e.* the addition-products must have the constitution represented by the formulæ:

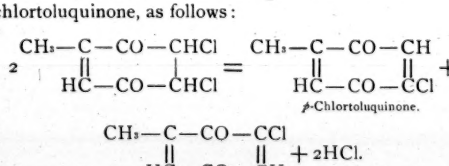


Toluquinone dichloride.



Toluquinone dibromide.

It is evident then that the dichloride, for instance, can lose one molecule of hydrochloric acid and give a mixture of meta- and parachlortoluquinone, as follows:



p-Chlortoluquinone.

m-Chlortoluquinone.

[1] This Journal **13**, 423.

Preparation of Quinones.—In the work described below, the ordinary methods of preparing quinones by oxidation with potassium (or sodium) dichromate were often found unsatisfactory. A simpler way to obtain these bodies, and one which is generally applicable, is to oxidise the corresponding hydroquinone, or often an amido-compound, by means of manganese dioxide and sulphuric acid in moderate excess. By brisk distillation with steam the quinone is quickly driven over, and can be at once obtained pure, and in very satisfactory quantity, from the well-cooled first portions of the distillate. When necessary, the filtrate, together with the less saturated portions of the distillate, can be extracted with ether in the usual way, and a small amount more of the substance obtained. The yield by this method is 50–60 per cent. of the calculated amount, and the troublesome emulsions and purifications of other processes are avoided. As this is by far the best way to obtain benzoquinone in quantity, and is frequently referred to below, the details of the process are given here.

Benzoquinone.—Upon the fresh mixture of 5 parts hydroquinone and 6 parts of powdered manganese dioxide, in a capacious flask, pour about 28 parts warm dilute sulphuric acid containing 7 parts ordinary acid. Mix well, and immediately distil briskly with steam, through a condenser, into a receiver kept as cold as possible by water containing salt and chopped ice. It is well to have the condenser warm at first to prevent clogging. Also, as the reaction at first is rather violent, the steam should be well under control, and the connection with the generator should be a loose one which can instantly be broken. The supersaturated part of the distillate is well cooled and the crystalline solid filtered off and dried by pressing well between bibulous paper. Fifty per cent. of the theoretical yield can thus be obtained in one to three hours. In the case of benzoquinone it is unprofitable to work up the ice-cold filtrate and the rest of the colored distillate, which together contain 5–10 per cent. more.

This method works well with relatively large quantities and is recommended for the manufacture of quinone. The difference in price between it and hydroquinone[1] should be greatly diminished thereby. On the large scale the saturated aqueous solution and the excess of manganese dioxide could be used for another lot.

[1] Kahlbaum (October, 1891) lists: quinone, 100 grams, 20 marks; hydroquinone, 100 grams, 2 marks.

I.—Benzoquinone Addition-products.

Quinone dichloride, $\begin{matrix} HC-CO-CHCl \\ \| \qquad\quad | \\ HC-CO-CHCl \end{matrix}$.—This substance is prepared as follows: 10 grams of quinone are dissolved in about 60 grams of dry chloroform in a 200-cc. flask, and into the solution, kept cold by surrounding it by ice and salt, is admitted a lively stream of dry chlorine. Moisture is excluded by stopping the flask with a doubly perforated cork, through one hole of which passes a wide tube, connected with the chlorine generator, and through the other an exit tube for escaping gases, protected at the top by calcium chloride. The chlorine is rapidly absorbed by the quinone solution, and in about ten minutes, during which the flask is shaken gently, crystalline quinone dichloride begins to separate out. After forty minutes the flask is detached and its contents washed with chloroform and dried on clay plates. If necessary, the product is purified by dissolving in fuming nitric acid and precipitating with water, but this step is usually superfluous.

Thus prepared, quinone dichloride is obtained as a pale yellowish-white, tasteless, nearly odorless, crystalline powder, melting at 146°[1] to a clear amber liquid, which decomposes in the capillary tube at about 185°. In the solid state it is very stable, subliming readily without decomposition. Recrystallised from glacial acetic acid it is obtained in thin, colorless, transparent plates. It is quite soluble in warm glacial acetic acid and in warm alcohol, but too much heating, especially in presence of water, decomposes it, hydrochloric acid being split off with ease. The light greenish-yellow solution, on warming with addition of a little water, darkens, and on cooling, fails to yield crystals. On extracting with ether, reddish-yellow rhombic crystals of monochlorquinone (melting-point 57°) are obtained:

$$\begin{matrix} HC-CO-CHCl \\ \| \qquad\quad | \\ HC-CO-CHCl \end{matrix} = \begin{matrix} HC-CO-CH \\ \| \qquad\quad \| \\ HC-CO-CCl \end{matrix} + HCl.$$

[1] The melting-points given in this paper, other than those quoted, may be regarded as "corrected." For those above 100° an apparatus enclosing the whole mercury column of a good thermometer, was employed, and all were frequently checked by comparative determinations with a standard set of Gerhardt's short thermometers. *e. g.* hydroquinone (melting-point 169°, uncorrected) melted in the apparatus used at 172°–172.5°; with Gerhardt's thermometer (145°–210°), at 172.5°–173°. The calculated correction was 3.7°.

The general action of reagents is likewise to split off hydrochloric acid. Benzene and acetone also dissolve it, and ether sparingly, but the safest solvent is chloroform, of which about twenty parts of hot, thirty at ordinary temperature, and a third more at 0°, are required. It dissolves without change in warm fuming nitric acid, and separates from concentrated solutions on cooling or diluting with water. Alkalies instantly decompose it like other halogen addition-products of quinone, forming a dark brown solution; the solid, when first touched with the alkali, turning dark bluish-green. Zinc and acetic acid convert it into hydroquinone.

Prepared as above, the yield of pure dichloride was uniformly 75–80 per cent. of the theoretical. Dried over sulphuric acid, it was analysed with the following results:

0.2098 gram substance gave 0.3068 gram CO_2 and 0.0426 gram H_2O.

0.2696 gram substance gave 0.4333 gram AgCl (Carius).

	Theory for $C_6H_4O_2Cl_2$.	Found.
C	40.22	39.89
H	2.23	2.25
Cl	39.67	39.76

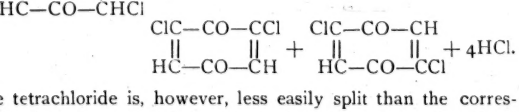
Quinone tetrachloride, $\begin{matrix} ClHC-CO-CHCl \\ | \qquad\quad | \\ ClHC-CO-CHCl \end{matrix}$.—This substance was prepared by dissolving quinone dichloride in about forty parts of dry chloroform, cooling and passing dry chlorine rapidly for 20–30 minutes, using the apparatus employed in the preparation of the dichloride. After standing some time in the cold, and rubbing the side of the flask with a glass rod, the tetrachloride begins to separate slowly. Standing at higher temperature hastens this formation somewhat, but also favors secondary reactions. In any case, a large quantity of hydrochloric acid is given off and the yield is small: *e. g.*, 8 grams of quinone dichloride dissolved in chloroform (300 grams) and treated with chlorine thirty minutes at 0°, on standing two hours in the cold and another hour at ordinary temperature, gave but 1.8 grams of tetrachloride. After washing with chloroform and drying on clay plates, the substance was treated with fuming nitric acid, washed, and again dried, finally over sulphuric acid in a vacuum.

0.2338 gram gave 0.2450 gram CO_2 and 0.0340 gram H_2O.
0.2520 gram gave 0.5708 gram AgCl (Carius).
0.1443 gram gave 0.3268 gram AgCl (Carius).

	Theory for $C_6H_4O_2Cl_4$.	Found. I.	II.
C	28.80	28.60	...
H	1.60	1.62	...
Cl	56.80	56.06	56.07

Quinone tetrachloride crystallises from chloroform in pure white microscopic prisms; recrystallised from glacial acetic acid, it occurs as colorless, transparent flat needles. It is odorless, and practically insoluble, in the cold, in ordinary solvents—glacial acetic acid, alcohol, ether, chloroform, and benzene dissolving it but sparingly. The small amount dissolved by hot fuming nitric acid is precipitated unchanged on diluting with water. Heated in a test-tube it sublimes, without melting, in rosettes of microscopic leaves or prisms; in a capillary it darkens slightly about 185°, and often sublimes without melting at 236°. Usually, however, it melts with decomposition, more or less sharply, at 226°, to a reddish-brown liquid.

On warming with dilute alcohol it loses hydrochloric acid, giving meta- and paradichlorquinone according to the equation:



The tetrachloride is, however, less easily split than the corresponding tetrabromide,[1] and as the hydrochloric acid split off can also react on the quinones formed, it is a somewhat delicate operation to accomplish the above change quantitatively. It was found best to boil for about 5 minutes and then pour into water and treat the precipitate, which contained unchanged tetrachloride, with a small amount of ether. The ethereal solution yielded on fractional crystallisation, two bodies. The less soluble one, melting at 157°, was paradichlorquinone; the more soluble, which was not yet quite free from the para-compound, melted at

[1] Nef: This Journal **12**, 486.

104°–106°, but was unquestionably metadichlorquinone[1] (melting-point 120°).

On treating quinone tetrachloride with zinc dust and acetic acid in the cold it is converted into pure hydroquinone.

Quinone dichloride-dibromide, $\begin{matrix} ClHC—CO—CHBr \\ | \quad\quad | \\ ClHC—CO—CHBr \end{matrix}$.—If to a cool concentrated solution of quinone dichloride in chloroform the calculated amount of bromine is added, and the mixture is allowed to stand in the cold, no reaction is apparent for some time. The color produced by the first drop of bromine persists and no rise of temperature is noticed. After a while, however, the color of the solution is appreciably lighter, and white flakes begin to separate, and continue to do so for an hour or more, if the solution is allowed to attain the temperature of the room, and the side of the flask is rubbed with a glass rod. Meanwhile, and for some hours after, no acid fumes make their appearance, and if the filtrate is set aside the dichloride-dibromide continues to crystallise out for several days, accompanied by but slight evolution of hydrobromic acid.

Quinone dichloride-dibromide was prepared in this manner, and, like the tetrachloride, washed with chloroform, treated with warm fuming nitric acid, washed with cold conc. nitric acid, then with water, and dried on clay plates. From 8 grams dichloride 4.6 grams were obtained.

0.3032 gram substance, dried over sulphuric acid in vacuum, gave 0.2389 gram CO_2 and 0.0315 gram H_2O.

0.2500 gram substance gave 0.4884 gram AgCl and AgBr (Carius).

	Theory for $C_6H_4O_2Cl_2Br_2$.	Found.
C	21.24	21.49
H	1.18	1.15
Cl	20.94 }	68.07
Br	47.20 }	

Thus prepared, quinone dichloride-dibromide is a white crystalline body melting at 202°–203° with decomposition, and subliming

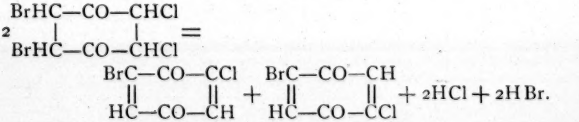
[1] The analogous decomposition has been carried out more carefully in the case of the dichloride-dibromide, and by Nef in case of the tetrabromide. The melting-point of the meta-compounds was always found low at first, owing to the presence of the para-product. To get it entirely free from the para-compound is a matter of much difficulty, requiring considerable material and repeated crystallisation. In this work, however, the melting-points invariably went up, and never was there a sign of a still lower melting ortho-compound. (*Cf.* also the behavior of meta- and para-chlor- and brom-toluquinones.)

to leafy rosettes. In solubility it is like the tetrachloride. It is decomposed on heating in dilute acetic acid or alcohol, but is stable in dry chloroform, being unchanged after standing three weeks in this solvent. Reduced with zinc and acetic acid, it is converted into hydroquinone.

Decomposition of Halogen Addition-products of Quinone by Heating in Solution.

As already stated, this decomposition is analogous in all cases. That of the dichloride-dibromide was the most carefully studied. 4.2 grams of this substance were warmed gently with common alcohol until a clear, amber-colored solution was obtained, a small amount of water (5–10 per cent.) was then added, and warming continued for a short time. On pouring the resulting brownish-yellow solution into 2–3 volumes of water a copious yellow precipitate was obtained which, filtered, washed with water and dried, weighed 2.2 grams. The filtrate and washings, on extracting with ether, gave a little more, making the yield a theoretical one. A low temperature seems essential, for, in other experiments, at boiling temperatures, secondary reactions apparently took place, yielding soluble products.

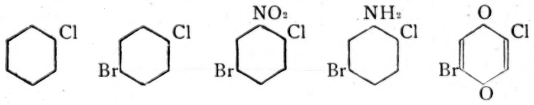
The yellow product dissolved with great ease in warm absolute ether, and on standing there were deposited two kinds of crystals which, by fractional crystallisation from a mixture of ordinary and petrolic ethers, were separated. The less soluble, compact crystals are identical with the chlorbromquinone obtained by Levy and Schultz.[1] The others, long straggly fibers, are metachlorbromquinone. Comparison of these products with the synthetical preparations (pp. 563 and 565) established their identity. A determination of the halogens in each by Carius' method gave 52.18 per cent. and 52.45 per cent. respectively, instead of the calculated 52.15 per cent. The following reaction has therefore taken place in the decomposition of quinone dichloride-dibromide:



[1] Ann. Chem. (Liebig) **210**, 160; Ber. d. chem. Ges. **15**, 656.

Synthetic Preparation of Parabromchlorquinone.

Since meta- and parabromchlorquinone have not yet been isolated, it was necessary to synthesise these two compounds and prove their identity with the decomposition-products of quinone dichloride-dibromide. The parabromchlorquinone was prepared from pure benzene by the following five steps:



I. II. III. IV. V.

The products I–III are known compounds.

Amidoparabromchlorbenzene.—Parabromchloraniline (IV) was prepared from the nitro-body (III)[1] by reduction with zinc and acetic acid. Ten grams nitroparabromchlorbenzene were dissolved in warm glacial acetic acid and 10–20 per cent. water added, but not in sufficient quantity to cause precipitation. To the hot solution was then added gradually, but as rapidly as was practicable, zinc dust, until the reaction was complete. The colorless solution was filtered hot, and the filtrate diluted and extracted with ether. After neutralising with sodium hydroxide, again extracting, and drying with stick potash, there separated on standing a considerable quantity of long, hard, yellowish-red needles in tufts and bunches. Other reduction-methods gave this azo-body as the chief product. It melts about 220° and is very sparingly soluble in water, ether, and alcohol. After filtering and distilling off the ether, the residue was distilled under diminished pressure. At about 170° under a pressure of 55 mm., 6 grams parachlorbromaniline distilled over, clear yellow at first, then slightly reddish, and solidified, on cooling, in a nearly white crystalline cake, while considerable of the azo-body remained behind.

Recrystallised from hot water (in which it is very sparingly soluble), fine, silky, white needles were obtained melting at 44.5°. Alcohol and ether dissolve the substance quite readily. Long standing with water decomposes it.

0.1980 gram, dried over sulphuric acid in a vacuum, gave 0.2514 gram CO_2 and 0.0478 gram H_2O.

0.2231 gram gave 0.3588 gram AgCl,AgBr (Carius).

[1] Körner: Jahresbericht, 1875, 327.

	Theory for $C_6H_3ClBr(NH_2)$.	Found.
C	34.87	34.63
H	2.42	2.68
Cl, Br	55.93	56.03

Hydrochloride of parabromchloraniline, $C_6H_3ClBr(NH_2),HCl+H_2O$.—The free base, dissolved in hot hydrochloric acid, yielded on standing, inch-long prisms with rhombic section, melting at 196°. The hydrochloride is less soluble in alcohol than the free base and far more soluble in hot water, but much heating with water partially decomposed it. A determination of HCl by precipitating AgCl from the aqueous solution, acidified with nitric acid, gave 14.10 per cent. HCl. Theory for the salt with one molecule crystal water, 13.99 per cent. (0.3685 gram substance gave 0.2032 gram AgCl.)

Sulphate of parabromchloraniline, $[C_6H_3ClBr(NH_2)]_2,H_2SO_4$. This salt crystallised from a hot solution of the base in dilute sulphuric acid in white ill-formed prisms or flat needles. Dried over sulphuric acid, dissolved in hot water containing hydrochloric acid, and digested several hours with barium chloride in slight excess, 0.1335 gram of the substance gave 0.0612 gram $BaSO_4$, corresponding to 19.27 per cent. H_2SO_4, instead of 19.18 per cent. required by theory.

Parabromchlorquinone.—After an unsatisfactory attempt to oxidise the amidoparabromchlorbenzene with sodium dichromate according to Nietzki's method for preparing ordinary quinone,[1] the following procedure gave excellent results. To 5 grams of the parabromchloraniline dissolved in about 150 cc. hot dilute sulphuric acid, 40 grams manganese dioxide were added and the mixture immediately distilled with steam. A beautiful yellow quinone-like body at once separated from the distillate, clogging the tube of the condenser, and more was obtained by extracting the distillate with ether.

This body is identical with one obtained from heating quinone dichloride-dibromide with dilute alcohol (see p. 560), as is shown by comparing the properties of the two.[2] The decomposition-product melted at 174°, the synthetic at 175°. Both have the same

[1] Ber. d. chem. Ges. **19**, 1467.

[2] It follows also that the product obtained by Levy and Schultz (*loc. cit.*) by treating chlorquinone with hydrobromic acid is *para*bromchlorquinone, since it corresponds in its properties with the above products.

orange-yellow color and pungent, musty odor. They are soluble in ether, less in ligroin, alcohol, and water, and more in chloroform, from which they crystallise in small rhombic plates. These, optically, are strongly doubly refractive and exhibit a remarkable pleochroism (yellowish-white, dark yellow). They belong to the monoclinic system. The acute angle of the rhombic face was measured approximately and found to be, in both, 56°–57°.

Analysis of synthetic (I) and decomposition (II) products:

I. *a.* 0.2203 gram synthetic product gave 0.2646 gram CO_2 and 0.0203 gram H_2O.

I. *b.* 0.2160 gram synthetic product gave 0.3240 gram AgCl, AgBr, which by reduction gave 0.2107 gram Ag.

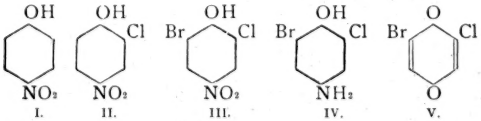
II. 0.2102 gram decomposition-product gave 0.3148 gram AgCl,AgBr, which by reduction gave 0.2044 gram Ag.

Theory for Ag in AgCl,AgBr found is 0.2110 and 0.2049 gram respectively.

	Theory for $C_6H_2ClBrO_2$.		Found.	
		I*a*.	I*b*.	II.
C	32.51	32.76	...	...
H	0.90	1.02	...	...
Cl	16.03			
Br	36.12	52.15	...	52.26	52.18

Synthetic Preparation of Metabromchlorquinone.

Starting with paranitrophenol of Kahlbaum's manufacture, the steps in this synthesis are represented by the following formulæ:



I. II. III. IV. V.

Chlornitrophenol (II) was prepared by chlorinating paranitrophenol in an exhausted flask with potassium chlorate.[1] The melting-point (111°) and other properties corresponded to those given by Kollrepp.

Metabromchlorparanitrophenol (III).—A solution of chlornitrophenol in warm glacial acetic acid was diluted with an equal volume of water, and to it, while constantly shaking, the calculated amount of bromine was slowly added. The bromine was

[1] Kollrepp: Ann. Chem. (Liebig) 234, 3.

readily absorbed, and when about half had been added, white crystals appeared and increased in quantity to the end. These were filtered off, dried on clay plates and then at 100°. They were practically pure, melted at 139.5°, and when analysed gave the following figures:

0.2206 gram substance gave 0.2270 gram CO_2 and 0.0250 gram H_2O.

0.2530 gram substance gave 0.3347 gram AgCl,AgBr (Carius).

	Theory for $C_6H_3BrClNO_2OH$.	Found.
C	28.51	28.06
H	1.19	1.26
Cl Br	45.74	46.08

Metabromchlorparaamidophenol (IV).—The above nitro-body, reduced with tin and conc. hydrochloric acid, gave a tin double salt of the corresponding amido-compound, which separated out almost completely from the concentrated acid solution, on cooling, in long, silky, white needles melting at 228°. It was filtered off, dried, dissolved in water, and the tin precipitated by hydrogen sulphide. The hydrochloride was converted into the sulphate by addition of sulphuric acid and evaporation. The latter salt crystallises in silky, white needles soluble in hot but nearly insoluble in cold water, and melts at 221°. The analysis gave unsatisfactory results, as only 16.50 per cent. H_2SO_4 was obtained, instead of 18.05 demanded by theory. The free base was therefore isolated by adding sodic carbonate to the hot aqueous solution of the sulphate. It came down in brownish-white prisms, and after recrystallisation from benzene was obtained in long, colorless needles. The analysis, however, gave low results in carbon, which, possibly, may be due to the fact that the substance burns with difficulty. No further work was done in this direction, as the product was not an essential, but simply an intermediate one.

Metabromchlorquinone (V).—The oxidation of metabrom-chlorparaamidophenol was effected, as in the para-series, by adding 8 parts manganese dioxide to a solution of the sulphate in dilute sulphuric acid and immediately distilling with steam. The yield is good, and the larger part of the product separates from the cooled distillate in soft masses of yellow needles with the characteristic odor.

This product resembles in every respect one obtained by heating the quinone dichloride-dibromide except that the latter melted 0.5°–1° lower, namely, about 114°. The synthetic product melted at 114°–115°. Both have a pungent, musty odor and crystallise from hot water in sulphur-yellow, fibrous needles. They sublime on heating and are very volatile with steam. They are very soluble in ether, less so in ligroin, alcohol, and water.

The following figures were obtained from analysis:

I*a*. 0.3054 gram synthetic product gave 0.3612 gram CO_2 and 0.0249 gram H_2O.

I*b*. 0.2232 gram synthetic product gave 0.3354 gram AgCl, AgBr (Carius).

I*c*. 0.2367 gram synthetic product gave 0.3558 gram AgCl, AgBr (Carius),—

which, reduced by hydrogen, gave $\left\{\begin{matrix}0.2165\\0.2300\end{matrix}\right\}$ gram Ag.

Theory for these quantities AgCl,AgBr $\left\{\begin{matrix}0.2185\\0.2318\end{matrix}\right\}$ gram Ag.

II. 0.2192 gram decomposition-product (p. 560) gave 0.3300 gram AgCl,AgBr and 0.2165 gram Ag.

(Theory: 0.2150 gram Ag.)

	Theory for $C_6H_2ClBrO_2$.	Found. Ia.	Ib.	Ic.	II.
C	32.51	32.26	...	...	...
H	0.90	0.91	...	...	...
Cl Br	52.15	...	52.35	52.37	52.45

II.—Toluquinone Addition-products.

Preparation of toluquinone.—Several attempts to obtain this body from orthotoluidine by oxidising it with potassium dichromate[1] gave unsatisfactory results, owing to the persistent emulsion which forms when the oxidised mixture is shaken with ether, and to the impurity of the extracted product.

A fair yield of pure substance can be quickly obtained by oxidising with manganese dioxide in the manner described above (page 555). On passing steam into a mixture of 140 grams manganese dioxide and 40 grams orthotoluidine dissolved in dilute sulphuric acid (160 grams acid and 480 grams water), contained in a flask capable of holding three or four liters, toluquinone will

[1] Schniter: Ber. d. chem. Ges. **20**, 2282.

pass over with a small quantity of distillate, and crystallise out, on cooling, in yellow plates melting at 68°–69°. By extracting the filtrate from this product, and the later portion of the yellow distillate, with ether, drying the ethereal solution with calcium chloride, and distilling at a low temperature, or quickly driving off the ether in the cold by a stream of dry air, about one-fourth more can be obtained.

The quantities given yielded 13.3 grams pure toluquinone in about an hour and 3.2 grams more on extracting. Should a portion of the contents of the flask be carried over by the vigorous frothing which sometimes takes place, the whole distillate can be treated with ether, but if the substances are well mixed and the steam is under control this can be prevented.

Toluquinone dibromide, $\begin{matrix} CH_3-C-CO-CHBr \\ \| \qquad\quad | \\ HC-CO-CHBr \end{matrix}$. — If to a cold solution of toluquinone in about three parts dry chloroform the calculated quantity of bromine is added, decoloration of the bromine takes place immediately, without evolution of hydrobromic acid. Spontaneous evaporation from a wide dish leaves a mass of light-yellow, flat needles of toluquinone dibromide. When dry these turn green, but washing with small quantities of alcohol leaves the body yellow. Also by dissolving in a mixture of concentrated and fuming nitric acids and pouring into ice-water, a light-yellow, crystalline powder is obtained which is pure. The yield is about 70 per cent.

0.2694 gram substance, dried over sulphuric acid in a vacuum, gave 0.2932 gram CO_2 and 0.0512 gram H_2O.

0.3150 gram substance gave 0.4212 gram AgBr (Carius).

	Theory for $C_7H_4O_2Br_2$.	Found.
C	29.78	29.68
H	2.13	2.11
Br	56.74	56.90

Toluquinone dibromide closely resembles benzoquinone dibromide[1] in appearance and properties, but it is more stable. It melts at 61°–62°, and sublimes at a higher temperature. It dissolves in alcohol, ether, chloroform, acetic acid, and somewhat in ligroin. Slowly soluble in ordinary nitric acid, the fuming acid dissolves it quite freely, and water precipitates the greater part

[1] Nef: This Journal **12**, 483.

from either solution. Heated in dilute alcohol or acetic acid solution, it breaks down into meta- and parabromtoluquinone (see below). This proves the constitution of the dibromide to be as given above. Reduced in the cold with zinc dust and acetic acid, it is converted into hydrotoluquinone (melting-point 125°).

On treating the dibromide in the cold with the theoretical quantity of bromine, no change was perceptible after twenty hours. Another mixture of the dibromide and bromine, with a little iodine added, was boiled with an inverted condenser for some time before any action took place, then copious acid fumes were evolved and a white solid separated, which, filtered off and crystallised from alcohol, melted at 203°. Oxidised by nitric acid, this gave a yellow, quinone-like body melting at about 234°. These bodies are probably tribromhydrotoluquinone and tribromtoluquinone respectively. Canzoneri and Spica,[1] by the action of bromine, iodine and *water* on toluquinone, obtained tribromtoluquinone mixed with a little dibromtoluquinone (melting-point 83°). They give as the melting-point of the first 235°, and of the hydroquinone that they got from it, 201°–202°. In this case the formation of quinones was doubtless due to the presence of water and the consequent oxidising action of the bromine.

Toluquinone dichloride, $\begin{matrix} CH_3—C—CO—CHCl \\ \| \qquad\quad | \\ HC—CO—CHCl \end{matrix}$, was made in the same manner as benzoquinone dichloride, by treating a solution of toluquinone in about four parts of dry chloroform, in the cold, with dry chlorine gas; but the reaction is slower and often incomplete, consequently the yield was usually not so great. By recrystallising from hot chloroform several times, a pure product was obtained, which, dried in a vacuum-desiccator over sulphuric acid and analysed, gave the following results:

0.1627 gram gave 0.2578 gram CO_2 and 0.0453 gram H_2O.

0.2387 gram gave 0.3566 gram AgCl (Carius).

	Theory for $C_7H_6O_2Cl_2$.	Found.
C	43.52	43.21
H	3.11	3.09
Cl	36.79	36.93

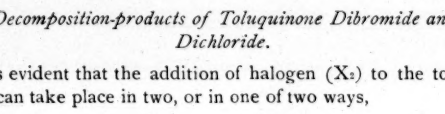
This body is perfectly analogous to its lower homologue. Its greater stability, however, allows it to be purified by repeated

[1] Ber. d. chem. Ges. **16**, 793.

crystallisation from hot chloroform. In this case also the chlorine-saturated solution from which the toluquinone dichloride first separated, can be made to yield more by direct evaporation. Evaporation of the benzoquinone-dichloride filtrate, on the other hand, causes substitution of the chlorine, and a complex mixture of solids is left. On boiling it in dilute alcoholic or acetic-acid solution, toluquinone dichloride is converted into meta- and para-chlortoluquinone (see below). This proves the constitution of the substance to be as given above. Reduced with zinc and acetic acid, it yields hydrotoluquinone. It is a cream-white, crystalline body, which melts at 135°–136° to an amber-yellow liquid, and decomposes in the capillary tube at about 164°. Heated in a test-tube, it sublimes apparently unchanged, like the di- and tetra-chloride of quinone.

Decomposition-products of Toluquinone Dibromide and Dichloride.

It is evident that the addition of halogen (X_2) to the toluquinone can take place in two, or in one of two ways,



where *A* represents an addition to the ethylene side of the molecule, and *B* to the propylene side. The addition-product *B* can lose halogen-hydride (HX) in but one way, and must then form an ortho-halogenated toluquinone, whereas the product *A* can lose halogen hydride in two ways, and give a meta- and a para-halogenated toluquinone (see reaction p. 554). It becomes thus an easy matter to determine the constitution of the toluquinone dibromide and dichloride described above; it is simply necessary to determine the nature of the decomposition-products.

1. *Toluquinone dibromide.*—The substance was dissolved in 85 per cent. alcohol and warmed for about 10 minutes on a water-bath, or till the solution turned brownish red. On pouring into two volumes of water a copious separation took place, either of yellow flakes or of a brownish oil which soon solidified. The yield was almost a theoretical one. On crystallising from water, alcohol, or from a mixture of ether and ligroin, long, prismatic yellow needles were obtained. Although more or less rough

under the microscope, these appeared perfectly homogeneous and melted sharply at 68°; there were left, however, a few unmelted particles. Repeated crystallisation from ligroin soon made it apparent that two substances were present; and by mechanical separation, thin, flat needles melting at 94°–95°, and compact, prismatic crystals melting at 105°–106°, were finally obtained. These melting-points correspond closely with those given for meta- (93°)[1] and for Schniter's bromtoluquinone (105°).[2]

A pretty sharp separation of the two isomers formed was at last accomplished by converting the mixture into the corresponding hydrotoluquinones. Parabromhydrotoluquinone was found to be far less soluble in water and in a mixture of ether and ligroin, than the meta-compound. Both are already known: the melting-point of the latter is given as 112°,[1] that of the former about 160°.[3] Reduction was effected by means of warm aqueous sulphurous acid, and the resulting light-yellow solution gave, on standing over night, white fibrous crystals like sal ammoniac. These, repeatedly crystallised from a mixture of ether and ligroin, gave a substance melting well at 176° (instead of 160.° See footnote below). This is parabromhydrotoluquinone. Oxidised with ferric chloride it gives parabromtoluquinone (melting-point found, 107°), and it behaves like the product obtained from the action of hydrobromic acid on toluquinone. The above aqueous solution extracted with ether, as well as the ether-ligroin mother-liquors from the para-compound, gave white leaflets which, though persistently recrystallised, melted at about 108°. On oxidising with ferric chloride, however, beautiful yellow needles melting at 94°–95° were obtained. It follows, therefore, that the product is metabromhydrotoluquinone. As Claus and Jackson give as the melting-point for this body 112°, it is highly probable that the above substance still contained traces of the para-compound, which it was not possible, in this case, to completely eliminate (*cf.* footnote, p. 559).

[1] Claus and Jackson: J. prakt. Chem. [2] **38**, 326–7.

[2] Ber. d. chem. Ges. **20**, 2286.

[3] Ibid. The body obtained by Schniter was, without doubt, impure parabromhydrotoluquinone, but the melting-point he gives is nearly 20° too low. His substance may have contained a little metabromhydrotoluquinone or gummy matter (*cf.* p. 573). On repeating his experiment at 0° (allowing aqueous hydrobromic acid to act on toluquinone for two days) there was obtained a light-colored body, which, crystallised from a mixture of ether and ligroin, gave a nearly white substance *melting sharply at* 179° with decomposition. The phenomena of this experiment were analogous to those observed when toluquinone was treated with hydrochloric acid (p. 573). In both cases the para-products thus obtained melted slightly higher than the reduction-products to be described.

The two hydroquinones, thus separated, were dried over sulphuric acid and their halogen contents determined by Carius' method.

I. 0.2898 gram of the para-compound gave 0.2673 gram AgBr.

II. 0.2067 gram of the meta-compound gave 0.1929 gram AgBr.

	Theory for $C_7H_7BrO_2$.	Found. I.	II.
Br	39.41	39.25	39.71

2. *Toluquinone dichloride.*—This substance was heated in dilute alcoholic solution exactly as above in the case of the corresponding dibromide. On adding water, an almost quantitative yield of isomeric chlortoluquinones was obtained which could not be separated from each other. An analysis of the mixture, crystallised from ligroin and melting at 68°–86°, gave figures agreeing well for a chlortoluquinone.

0.1741 gram substance, dried over sulphuric acid, gave 0.1601 gram AgCl (Carius).

	Theory for $C_7H_5ClO_2$.	Found.
Cl	22.69	22.75

The separation of this mixture into its constituents was accomplished by means of the hydroquinones, exactly as in the case of the corresponding brom-compounds. In this way, thin, white needles melting at 172°–173°, and leaflets in bunches which melted at 105°–107°, were obtained. These correspond to para-[1] and to metachlorhydrotoluquinone[2] respectively, but the same difficulty was experienced here as in case of the brom-compounds, in getting an absolutely pure meta-product.

The two chlorhydrotoluquinones obtained were dried over sulphuric acid and the amount of chlorine in each was determined by Carius' method.

I. 0.1780 gram of para-body gave 0.1613 gram AgCl.

II. 0.1630 gram of meta-body gave 0.1482 gram AgCl.

	Theory for $C_7H_7ClO_2$.	Found. I.	II.
Cl	22.39	22.41	22.48

III.—The Action of Haloid Acids on Quinones.

From the action of halogens on quinones, as shown above and in a previous paper by Nef,[3] it seemed highly probable that the

[1] Schniter (*loc. cit.* p. 2285) gives melting-point of his product as about 175°. *Cf.* p. 574 below.

[2] Melting-point 115°: Claus and Jackson (*loc. cit.* p. 328).

[3] This Journal **12**, 483.

action of haloid acids on quinones takes place in a similar manner. The ease with which quinone, for instance, forms a dibromide leads one to expect that it might form, with hydrobromic acid, a hydrobromide, $HC-CO-CHBr$, especially since olefine deriv-

$$\begin{matrix} \| & & | \\ HC-CO-CH_2 \end{matrix}$$

atives, as is well known, take up halogen hydrides with almost the same ease as halogens. Notwithstanding this, however, it can be shown that the behavior of quinones towards halogens and halogen hydrides is totally different.

My own experiments on the action of haloid acids on quinones are simply confirmatory of the results already obtained by Sarauw[1] and by Levy and Schultz.[2]

The Action of Hydrochloric Acid on Benzoquinone.

On passing *dry* hydrochloric acid into a 5-per cent. solution of quinone in dry chloroform, after two minutes much quinhydrone has separated out, and after about twenty minutes the mass is perfectly colorless, consisting of practically pure chlorhydroquinone melting at 106° and in theoretical amount. An entirely analogous result is obtained by using dry hydrobromic acid, except that in this case the resulting colorless mass is a mixture of bromhydroquinone (chief product) and para-dibromhydroquinone.

That the product formed at first in these two cases is quinhydrone was proved by filtering it off, washing well with chloroform and treating with hydrochloric acid. Although a Carius determination always gave 1–3 per cent. of halogen in this intermediate product, when suspended in chloroform and treated with dry hydrochloric acid it gave a mixture of nearly equal parts of chlorhydroquinone and hydroquinone. These results show that in the action of hydrochloric acid on quinone, one molecule of chlorine is set free according to the reaction:

$$\text{I. } C_6H_4O_2 + 2HCl = C_6H_4(OH)_2 + Cl_2.$$

The resulting hydroquinone, before it can be acted upon by the chlorine set free, unites immediately with the quinone present in excess, forming quinhydrone, $C_6H_4(OH)_2,C_6H_4O_2$. The further action of the hydrochloric acid is to act on the quinone portion of the quinhydrone molecule, converting it into hydroquinone, and another molecule of chlorine is set free:

$$\text{II. } C_6H_4(OH)_2,C_6H_4O_2 + 2HCl = 2C_6H_4(OH)_2 + Cl_2.$$

[1] Ann. Chem. (Liebig) **209**, 99. [2] *Ibid.* **210**, 133.

The chlorine set free in this last case (II) is only sufficient to convert one-half of the hydroquinone formed into chlorhydroquinone. Now pure quinhydrone when treated with hydrochloric acid is converted into equal molecules of hydroquinone and chlorhydroquinone. The same is true of the above intermediate product if it is filtered off from the chloroform solution, and consequently the solution must contain free chlorine (one molecule).

It follows therefore that quinone oxidises the hydrochloric acid, taking up its hydrogen to form hydroquinone and setting free chlorine. A number of new observations made by Dr. Nef[1] suffice to clear up this remarkable and enigmatic behavior of benzoquinone towards halogen hydrides.

Benzoquinone shows a twofold chemical behavior, owing to the fact that it contains, first, two ethylene groups, $\begin{matrix}CH—\\ \|\\ CH—\end{matrix}$, which are reactive towards halogens; and secondly, two carbonyl groups, $\begin{matrix}O\\ \|\\ —C—\end{matrix}$, which are reactive towards halogen hydrides and water.

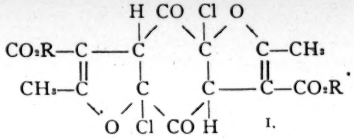
The action of hydrochloric acid on quinone, *e. g.*, is in the first place to form the addition-product $\begin{matrix}Cl\ \ OH\\ \diagdown\ \ /\\ HC—C—CH\\ \|\ \ \ \ \|\\ HC—C—CH\\ /\ \ \diagdown\\ Cl\ \ OH\end{matrix}$, which immediately loses chlorine (one molecule), giving hydroquinone. The chlorine set free then reacts further on hydroquinone, giving chlorhydroquinone and hydrochloric acid.

That the above explanation is correct is made still more probable by some experiments since carried out in this laboratory by Dr. M. Ikuta. He had formerly obtained, by treating paradichlorquinone diaceto acetic ether,

$$\begin{matrix}&&&&CO&&&&\\ &&&&/\ \ \diagdown&&&&\\ CO_2R—C&———&C&&CCl&&COH—CH_3&&\\ \|&&\|&&\|&&\|&&\\ CH_3COH&&ClC&&C&———&C—CO_2R&&\\ &&&&\diagdown\ \ /&&&&\\ &&&&CO&&&&\end{matrix},$$

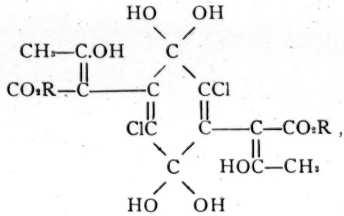
[1] Ann. Chem. (Liebig) **270**, 324.

with dilute sulphuric acid,[1] an isomer which he regarded as quinone-α-dimethyldifurfuran-β-dicarboxylic ether dihydrochloride,



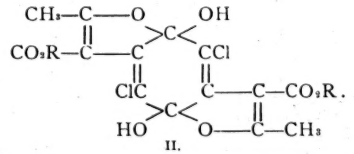
I.

It was supposed that two molecules of water had added themselves to the ethylene group in the quinone. In view of the results obtained by Nef it seems probable that the addition of water takes place at the carbonyl groups, as follows:



Addition-product.

which loses two molecules of water,



II.

The results obtained thus far point entirely towards formula II. because the substance dissolves unchanged in sodic hydrate.

The Action of Hydrochloric Acid on Toluquinone.

Schniter[2] has shown that, on treating toluquinone with concentrated hydrochloric acid, a chlorhydrotoluquinone is formed which melts at 175°. I also have studied the action of strong

[1] Jour. prakt. Chem. [2] **45**, 75. [2] Ber. d. chem. Ges. **20**, 2285.

aqueous hydrochloric acid on this quinone and found that besides the para-compound, melting at 174–6°, some metachlorhydrotoluquinone is formed. Since in this reaction much gummy matter appears, more satisfactory results were obtained by passing *dry* hydrochloric acid into a cold 15-per cent. solution of toluquinone in chloroform, whereby the same phenomena were observed as in the case of quinone. On evaporating the chloroform and purifying the white residue by fractional crystallisation, first from water containing sulphurous acid and then from ligroin, much parachlorhydrotoluquinone (melting-point 176°) and some of the meta-compound (melting-point found 106–7°) were obtained. These products are identical with those described above (p. 570), and the same difficulty was experienced in isolating the more soluble body.

I. 0.2601 gram of the para-compound, dried over sulphuric acid, gave 0.2338 gram AgCl (Carius).

II. 0.1651 gram meta-compound gave 0.1505 gram AgCl.

	Theory for $C_7H_7ClO_2$.	Found.	
		I.	II.
Cl	22.39	22.23	22.54

IV.—On Quinhydrones.

A few observations on quinhydrones were made that may possibly be of interest. Quinhydrone itself separates out in at least 91-per cent. yield on mixing ethereal or aqueous solutions of quinone and hydroquinone in equivalent amounts. In the former case it comes down in the form of dark green, irregular plates; in the latter, as bronze-green needles. All solvents dissociate it to some extent, especially those in which the two constituents are unequally soluble, as, for instance, chloroform. This is readily shown by the fact that the dry quinhydrone, which is odorless, instantly smells strongly of quinone when moistened with a solvent. On treating quinhydrone, suspended in dry chloroform, with dry hydrochloric acid until the mass is colorless, a mixture of equal molecules of hydroquinone and chlorhydroquinone results. These are readily separated by means of chloroform. In an experiment, 91 per cent. of the latter and 97.5 per cent. of the former substance was obtained.

Chlorquinhydrone.—Wichelhaus[1] obtained quinhydrone, and never a substituted quinhydrone, on bringing together, either

[1] Ber. d. chem. Ges. **12**, 1501–3.

quinone and a chlorinated hydroquinone, or hydroquinone and a chlorinated quinone. Chlorquinones, on the contrary, give chlorinated quinhydrones with their corresponding chlorhydroquinones[1]—in other words, no derivative of quinhydrone, $C_{12}H_{10}O_4$, containing an odd number of chlorine atoms is known.

The results which I have obtained in this direction seem to show a great difference according to whether quinone and a chlorinated hydroquinone be taken, or hydroquinone and a chlorinated quinone. In the former case a very complicated reaction sets in, whereas in the latter instance a chlorinated quinhydrone is actually formed.

Quinone and chlorhydroquinone.—On mixing chloroform solutions of these two substances (equal molecules), bronze-green scales separated out which, well washed, contained various quantities of chlorine but never over 3.5 per cent. Suspended in chloroform and treated with hydrochloric acid, these were converted into a mixture of hydroquinone (90 per cent.) and chlorhydroquinone (80 per cent.), and this proves that the product consisted chiefly of quinhydrone. The amount formed, however, was small, and another experiment was carried out with larger quantities and with absolute ether as the solvent. On mixing the ethereal solutions an immediate darkening was noticed, but no further change for an hour, and even after five hours' standing only a very slight amount of dark green needles had separated out. After three days the amount of this material had increased, but on filtering, it was found to be only one-fifth of the total possible. The mass obtained was of a dull, greenish-black color, and on analysis gave 6.46 per cent. chlorine. The ethereal filtrate when concentrated gave more of a similar substance, but in no case was the indication of a homogeneous substance at hand.

In order to determine the nature of the mixture, steam was passed into it and the quinones carried over. The residue contained, besides hydroquinones, much tarry matter which made the separation of the former a very difficult matter; but finally, by fractional crystallisation from benzene, the presence of much chlorhydroquinone and some hydroquinone was established. The volatile portion gave quinone in considerable amount and some chlorquinone.

[1] Staedeler: Ann. Chem. (Liebig) **69**, 308, 314.

These results show that the action of quinone on chlorhydroquinone is very slow and complicated in its nature.

Chlorquinone and hydroquinone.—A marked difference is noticed here, compared with the case just mentioned. On mixing ethereal solutions containing equivalent quantities of chlorquinone and hydroquinone, the separation of a solid, although not immediate as in the case of quinhydrone (p. 574), begins after four or five minutes and continues uniformly. After twenty minutes, 50 per cent. of the entire material used was obtained by filtering and washing with a small quantity of ether. The substance obtained consisted of bronze-yellow, metallic needles, which appeared perfectly homogeneous. A determination of the halogen, according to Carius, gave 13.08 per cent. chlorine; theory for a monochlorquinhydrone, $C_{12}H_9ClO_4$, being 14.06 per cent. The somewhat low percentage of chlorine found may be due to a slight dissociation of the quinhydrone in the washing with ether. At any rate, this substance appears to be as easily split as ordinary quinhydrone, and as the more soluble constituent in this case is chlorquinone, it would be removed in larger quantity. This explanation is the more plausible because, on washing the material obtained with larger quantities of ether, the amount of the halogen constituent of the substance left was lessened.

The results thus point to the existence of a quinhydrone of the formula $C_6H_4(OH)_2,C_6H_3ClO_2$.

THE CONDENSATION-PRODUCTS OF ACETACETIC-ETHER HYDRAZIDE AND OF OXALACETIC-ETHER HYDRAZIDE.

By Charles Walker.

That acetacetic ether reacts with phenylhydrazine, forming a hydrazide of the constitution $\begin{matrix} CH_3.C.NH.NH.C_6H_5 \\ \| \\ H.C.CO_2.C_2H_5 \end{matrix}$, has been proved with all precision by the experiments of Nef.[1] This hydrazide, according to the conditions employed, can undergo various condensations: *e. g.*, (*a*) Knorr[2] has shown that it readily

[1] Ann. Chem. (Liebig) **266**, 74.

[2] *Ibid.* **237**, 147.

loses alcohol (one molecule), forming (1)phenyl(3)methyl(5)pyrazolon,[1] $\begin{matrix}CH_3.C{-}NH\\ \|\quad\quad\\ HC{-}CO\end{matrix}\!>N.C_6H_5$. (*b*) Nef has shown that when treated with concentrated sulphuric acid it loses one molecule of ammonia, forming an indol derivative.[2] I have continued the study of this subject under the direction of Dr. Nef, and have found that the indol derivative formed is ethylic *α*-methylindol-*β*-carboxylate. (*c*) Furthermore, it was found that acetacetic-ether hydrazide, under still different conditions, reacts yet in another way, losing one molecule of water, as follows:

$$\begin{matrix}CH_3.C.NH.NH.C_6H_5\\ \|\\ H.C.C{=}O\\ |\\ O.C_2H_5\end{matrix} \quad = \quad \begin{matrix}CH_3.C{=}N\\ |\quad\quad\\ HC{=}C\end{matrix}\!>\!\begin{matrix}N.C_6H_5\\ \\ O.C_2H_5\end{matrix} + H_2O,$$

forming thus (1)phenyl(3)methyl(5)ethoxypyrazol.

Entirely analogous results were obtained in the case of oxalacetic-ether hydrazide, $\begin{matrix}CO_2.C_2H_5.C.NH.NH.C_6H_5\\ \|\\ H.C.CO_2.C_2H_5\end{matrix}$, except that here the conversion into an indol derivative (case *b*) has not yet been accomplished.

Ethylic α-methylindol-β-carboxylate, $C_6H_4\!<\!\begin{matrix}C{-}CO_2.C_2H_5\\ \|\quad\quad\\ NH\quad C.CH_3\end{matrix}$.—It is evident that acetacetic-ether hydrazide can lose ammonia (one molecule) in one of two ways:

$$H_4C_6\!<\!\begin{matrix}NH{-}NH\\ H\end{matrix}\!>\!\begin{matrix}\\ C.CH_3\\ \|\\ H.C{-}CO_2R\end{matrix} = C_6H_4\!<\!\begin{matrix}NH\\ \\ C.CH_3\\ \|\quad\quad\\ C{-}CO_2R\end{matrix}\!> + NH_3 \qquad (I)$$

$$H_4C_6\!<\!\begin{matrix}NH{-}NH\\ H\end{matrix}\!>\!\begin{matrix}C{:}CH.CO_2R\\ H_3C\end{matrix} = C_6H_4\!<\!\begin{matrix}NH\\ CH_2\end{matrix}\!>C{:}CH.CO_2R =$$

$$C_6H_4\!<\!\begin{matrix}NH\\ \\ CH\end{matrix}\!>\!\begin{matrix}\\ C{-}CH_2.CO_2R\\ \|\quad\quad\\ =\quad\quad\end{matrix} + NH_3. \qquad (II)$$

Indol-α-acetic ether.

[1] Ann. Chem. (Liebig) **266**, 124. [2] *Ibid.* **266**, 73.

That this takes place exclusively according to reaction (I) was proved by the direct synthesis of ethylic α-methylindol-β-carboxylate: α-methylindol-β-carboxylic acid (melting-point found 176°) was made, according to Ciamician and Magnanini's[1] directions, from methylketol, and the acid converted into the silver salt. As the free acid loses carbon dioxide very readily, the salt was prepared by adding silver nitrate to a neutral solution of the ammonium salt in the cold. The white, flocculent precipitate thus obtained was well washed, dried in a vacuum, and, after moistening with ether, heated for 10 hours on a water-bath with twice the calculated amount of ethyl iodide. The ethyl ester thus obtained crystallised in colorless needles, melting at 131°, and was identical in all its properties with Nef's indol derivative.[2] I prepared the latter in large amounts according to Nef's directions, and obtained about a 30-per cent. yield. The crude product crystallises from alcohol, at first in scales, but, after repeated crystallisation, is obtained in lustreless needles. The melting-point is 131° and not 134°. The substance gives no coloration with ferric chloride. It is remarkably stable towards alcoholic potash, and can be heated for a day with a large excess without much change. If heated in a sealed tube, however, at 150° for 6 hours with twice the calculated amount of caustic potash, it is split quantitatively into methylketol and potassic carbonate. The ease with which the carboxyl group is split off here is noteworthy, because a number of indol derivatives, *e. g.* indol-α-carboxylic acid, have been obtained by melting indol homologues with caustic potash.[3] The methylketol formed was distilled over with steam, and obtained practically pure, melting at 56°–60° and showing all the characteristic reactions of this substance.[4] For the analysis it was recrystallised from ligroin (melting-point found 60°).

0.1817 gram substance gave 0.5462 gram CO_2 and 0.1150 gram H_2O.

0.2112 gram substance gave 20.1 cc. moist nitrogen at 25° and 746 mm.

	Theory for C_9H_9N.	Found.
C	82.44	81.99
H	6.86	7.03
N	10.69	10.70

[1] Ber. d. chem. Ges. **21**, 1925. [2] Ann. Chem. (Liebig) **266**, 73.
[3] Ciamician and Zatti: Ber. d. chem. Ges. **21**, 1938.
[4] Jackson: *ibid.* **14**, 879; Fischer: Ann. Chem. (Liebig) **236**, 127.

Ethylic (1)*phenyl*(5)*ethoxypyrazol*(3)*carboxylate*, $C_2H_5.O_2C{-}C{=}N$ / $HC{=}C.O.C_2H_5$ >$N.C_6H_5$.—Oxalacetic ether reacts with phenylhydrazine,[1] forming a substance which is to be regarded as oxalacetic-ether hydrazide,[2] $CO_2R.C.NH.NH.C_6H_5$ / $H.CO.O.C_2H_5$. This compound, as W. Wislicenus has shown, loses one molecule of alcohol very readily at higher temperatures, forming ethylic (1)phenyl(5)pyrazolon(3)carboxylate, $CO_2R.C{-}NH$ / $HC{-}CO$ >$N.C_6H_5$. It was expected that concentrated sulphuric acid would convert it, as analogous to the acetacetic-ether hydrazide, into ethylic indol-$a\beta$-dicarboxylate, with elimination of one molecule of ammonia, but this is not the case.

The oxalacetic-ether hydrazide was prepared by adding phenylhydrazine to oxalacetic ether (one molecule), taking care that the temperature does not rise above 50°; after standing for 2 hours, ether was added, and the ethereal solution washed with dilute sulphuric acid, and then dried with calcic chloride. The ether was distilled off at low temperature, and the last traces removed over sulphuric acid in a vacuum. The yellowish solid left was well washed by decantation with ligroin (boiling-point 70°–80°), and so an almost pure white oxalacetic-ether hydrazide obtained, melting at 76°–78°.

The hydrazide, when treated with dehydrating agents, loses one molecule of water, forming a neutral body, melting at 83°–84°. In all cases, however, ethylic (1)phenyl(5)pyrazolon(3)carboxylate and the free acid are the chief products formed; and these, being both acid in nature, can easily be separated from the neutral compound, by extracting with ether and shaking with sodic hydroxide.

The following yield of the new substance was obtained:

(*a*) On treating with concentrated sulphuric acid at 0° and, after 10 minutes' standing, pouring on ice, and extracting with ether, etc.:

7.7 grams hydrazide gave 0.7 gram.
3.5 grams hydrazide gave 0.4 gram.

[1] Ann. Chem. (Liebig) **246**, 319. [2] *Ibid.* **266**, 65.

(*b*) On treating an alcoholic solution at 0° with dry hydrochloric acid, warming gently to 60°, and then pouring into water and extracting, etc.:

2 grams hydrazide gave 0.2 gram.

(*c*) same as (*b*), except that acetic acid was used instead of alcohol:

2 grams hydrazide gave 0.2 gram.
2 grams hydrazide gave 0.3 gram.

The best yield was finally obtained (5 grams from 20 grams hydrazide) by saturating an acetic-acid solution of the hydrazide at 0° with dry hydrogen chloride, and then warming on a water-bath at 60° for an hour, pouring into water, etc. The ethereal solutions were dried with calcic chloride, and the yellowish-colored solid left after removing the ether was recrystallised from dilute alcohol until colorless. It was obtained in needles, melting at 83°–84°, and, after drying over sulphuric acid in a vacuum, gave the following result on analysis:

I. 0.2048 gram substance gave 0.4836 gram CO_2 and 0.1200 gram H_2O.

II. 0.3282 gram substance gave 0.7710 gram CO_2 and 0.1830 gram H_2O.

III. 0.3443 gram substance gave 34 cc. moist nitrogen at 18° and 757 mm.

IV. 0.1925 gram substance gave 18.4 cc. moist nitrogen at 20° and 755 mm.

	Theory for $C_{14}H_{18}N_2O_3$.	Found. I.	II.	III.	IV.
C	64.61	64.40	64.07	...	...
H	6.15	6.51	6.19	...	...
N	10.77	...	...	11.23	10.80

Oxalacetic-ether hydrazide, $C_{14}H_{18}N_2O_4$, has therefore lost one molecule of water; that the splitting off of water takes place thus:

$$\begin{array}{l} C_2H_5.CO_2.C{-}NH{-}N.C_6H_5 \\ \quad\| \qquad\quad H \\ H.C{-}CO \\ \quad\quad | \\ \quad\quad O.C_2H_5 \end{array} = \begin{array}{l} C_2H_5.CO_2.C{=}N \\ \quad\quad | \quad\quad >N.C_6H_5 \\ H.C{=}CO.C_2H_5 \end{array} + H_2O,$$

is proved by the further study of the resulting product. The new compound is very soluble in most organic solvents except ligroin; it is insoluble in water. It possesses weak basic prop-

erties, dissolving in concentrated acids, but on adding water the substance comes down unchanged. That it has an ethoxy group, as well as an ethylated carboxyl group, is proved by what follows.

(1)*Phenyl*(5)*ethoxypyrazol*(3)*carboxylic acid*, $HO_2C.C{=}N$ / $H.C{=}C.OC_2H_5$ $>N.C_6H_5$.—The ester just described is heated on a water-bath for 8 hours with twice the calculated amount (the same result is obtained if 10 times this amount is used) of alcoholic potash. After driving off the alcohol and taking up with water, dilute hydrochloric acid precipitates a greyish-white substance. Recrystallised twice from much boiling water, it is obtained in transparent plates or in flat needles, which become opaque on drying at 110° and melt at 152°–153°.

I. 0.2022 gram substance, dried at 110°, gave 0.4578 gram CO_2 and 0.0982 gram H_2O.

II. 0.2174 gram substance gave 0.4979 gram CO_2 and 0.1058 gram H_2O.

III. 0.1928 gram substance gave 20.2 cc. moist nitrogen at 11° and 738 mm.

	Theory for $C_{12}H_{12}N_2O_3$.	I.	Found. II.	III.
C	62.07	61.74	62.41	...
H	5.17	5.41	5.41	...
N	12.07	...	...	12.06

The acid is readily soluble in ether, chloroform, and alcohol; it dissolves with difficulty in boiling, and only very slightly in cold water. It gives no coloration with ferric chloride. A hydrochloride separates out in needles on dissolving the acid in a small amount of warm concentrated hydrochloric acid and cooling; it is decomposed by water. That the acid is monobasic is proved by the analysis of the barium and calcium salts.

Barium salt, $(C_{12}H_{11}N_2O_3)_2Ba.2H_2O$.—The barium salt was prepared by heating the acid with water and an excess of barium carbonate until neutral reaction sets in. After filtering and concentrating the salt crystallises out in colorless needles. The salt, after standing in a desiccator for 36 hours, lost two molecules of crystal-water on drying at 140°–150°.

0.9808 gram substance, dried 4 hours at 150°, lost 0.0292 gram H_2O.

	Theory.	Found.
H_2O	2.92	2.98

The analysis of the anhydrous salt gave the following result:

I. 0.2182 gram substance gave 0.3820 gram CO_2 and 0.0844 gram H_2O.

II. 0.2096 gram substance gave 0.0813 gram $BaSO_4$.

	Theory for $(C_{13}H_{11}N_2O_3)_2Ba$.	Found. I.	II.
C	48.08	47.76	...
H	3.67	4.29	...
Ba	22.87	...	22.81

Calcium salt, $(C_{13}H_{11}N_2O_3)_2Ca.4H_2O$.—The calcium salt was prepared by a method entirely analogous to that for the barium salt. It is more soluble in water than the latter and crystallises in rhombic plates, containing four molecules of crystal-water.

0.2360 gram substance, air-dried (14 hours), lost 0.0284 gram H_2O on heating to constant weight at 110°.

	Theory.	Found.
H_2O	12.54	12.04

The anhydrous salt gave the following result on analysis:

I. 0.1638 gram substance gave 0.3441 gram CO_2 and 0.0680 gram H_2O.

II. 0.0980 gram substance gave 0.0253 gram $CaSO_4$.

	Theory for $(C_{13}H_{11}N_2O_3)_2Ca$.	Found. I.	II.
C	57.37	57.29	...
H	4.38	4.61	...
Ca	7.97	...	7.57

(1) *Phenyl(5)oxypyrazol(3)carboxylic acid*, $\begin{matrix} HO_2C.C{=}N \\ \quad\quad\quad\

	Theory for $C_{10}H_8N_2O_3$.	Found.
C	58.82	58.90
H	3.92	4.42

This acid can naturally be obtained in one operation from the ethylic (5)ethoxypyrazol(3)carboxylate by heating in sealed tubes at 150° for two hours. Longer heating is harmful in all cases owing to the instability of the resulting acid.

Phenyloxypyrazolcarboxylic acid is very difficultly soluble even in boiling water and separates out in needles on cooling. The aqueous solution gives a purplish-blue coloration with ferric chloride. The substance is far less stable than the corresponding ethoxy acid: it loses carbon dioxide on heating to 230°, and although very nearly one molecule is split off in this way, the residue consists in great part of charred matter and not a trace of (1)phenyl(5)oxypyrazol was obtained. In attempting to make the barium and the calcium salt of the acid as above in the case of the ethoxy acid, it was found that on concentrating the solutions, deep reddish-violet coloration and complete decomposition sets in; this is also true of the ammonium salt.

A silver salt was, however, obtained by adding silver nitrate in the cold to the carefully neutralised solution of the ammonium salt. The salt comes down as a colorless, gelatinous precipitate, and the analysis shows that two atoms of hydrogen have been replaced by silver.

0.2660 gram substance, dried over H_2SO_4 in a vacuum, gave 0.2770 gram CO_2, 0.0446 gram H_2O and 0.1365 gram Ag.

	Theory for $C_{10}H_6N_2O_3Ag_2$.	Found.
C	28.70	28.40
H	1.43	1.86
Ag	51.63	51.32

(1)*Phenyl*(5)*ethoxypyrazol*, $\begin{matrix} HC{=}N \\ | \quad > N.C_6H_5 \\ HC{=}C.O.C_2H_5 \end{matrix}$—(1)Phenyl-(5)ethoxypyrazol(3)carboxylic acid, when heated to 205°, begins to lose carbon dioxide; one molecule is split off almost quantitatively, as shown by the following experiment:

10.71 grams substance, heated in a stream of dry air, first at 205° and then gradually up to 230°, lost 1.825 gram CO_2.

Theory for one molecule.	Found.
18.97	17.40

There is left a dark red-colored oil of weak basic properties. Attempts to distil it under reduced pressure showed that it does not boil without decomposition. It is not volatile with steam, and, if heated in a sealed tube with concentrated hydrochloric acid at 150°, is completely decomposed.

That the oil is phenylethoxypyrazol could, however, be proved by the isolation of a platinum double salt: the substance was dissolved in alcoholic hydrochloric acid, and the calculated amount of platinic chloride, dissolved in alcohol, added. After a short time the double salt begins to separate in yellow plates, which, recrystallised from alcoholic hydrochloric acid several times, are obtained in pale yellow needles melting at 141°–3°. For the analysis the salt was dried over sulphuric acid and lime in a vacuum.

I. 0.2654 gram substance gave 0.3243 gram CO_2 and 0.0998 gram H_2O.

II. 0.2981 gram substance gave 18.5 cc. moist nitrogen at 20° and 744 mm.

III. 0.2240 gram substance gave 0.0555 gram Pt.

IV. 0.2695 gram substance gave 0.2859 gram AgCl (Carius).

	Theory for $(C_{11}H_{12}N_2O, HCl)_2.PtCl_4$.	Found. I.	II.	III.	IV.
C	33.65	33.33	...	...	...
H	3.31	4.17	...	...	...
N	7.14	...	6.92	...	...
Pt	24.78	...	...	24.78	...
Cl	27.04	...	...	...	26.24

The Third Condensation-Product of Acetacetic-Ether Hydrazide.

(1)*Phenyl*(3)*methyl*(5)*ethoxypyrazol*, $\begin{matrix} CH_3.C{=}N \\ \quad\quad\quad\quad > N.C_6H_5.— \\ H.C{=}C.O.C_2H_5 \end{matrix}$

Freer[1] has recently published a brief notice of a new compound obtained by treating acetacetic ether with phenylhydrazine hydrochloride. He gives this substance the formula $C_{10}H_{10}N_2O$ and supposes it may be an isomer of Knorr's phenylmethylpyrazolon. His conclusions are based entirely on conjectures and are without the slightest experimental basis, as the analysis of the product does not agree at all well with the formula assumed.

[1] J. prakt. Chem. [2], **45**, 414.

In view of the above results with oxalacetic-ether hydrazide, it seemed highly probable that Freer's product was formed from acetacetic-ether hydrazide by the splitting off of one molecule of water, and not of alcohol, as Freer assumes—$C_{12}H_{16}N_2O_3 = H_2O + C_{12}H_{14}N_2O$ (*cf.* page 577).

Freer's condensation-product was therefore made according to his directions in considerable quantity. The resemblance in its properties to those of ethoxyphenyl pyrazol, obtained above, is a very close one. The substance does not distil under reduced pressure entirely without decomposition, and this explains the poor analyses of Freer, which agree about as well for one as the other formula. It forms, however, a fine crystalline platinum double salt, which is best obtained by passing dry hydrochloric acid in excess into an ethereal solution of the oil, and then adding the calculated amount of platinic chloride dissolved in alcohol. After standing for a time, the double salt separates out in reddish-yellow plates which, recrystallised once from alcoholic hydrochloric acid, are obtained in yellow six-sided prisms, melting at 191°. For the analysis the salt was dried over sulphuric acid and lime in a vacuum.

I. 0.2515 gram substance gave 0.3274 gram CO_2 and 0.0906 gram H_2O.

II. 0.1976 gram substance gave 0.2567 gram CO_2 and 0.0717 gram H_2O.

III. 0.3594 gram substance gave 22.5 cc. moist nitrogen at 27° and 750 mm.

IV. 0.2656 gram substance gave 17 cc. moist nitrogen at 23° and 750 mm.

V. 0.2732 gram substance gave 0.0645 gram Pt.

VI. 0.2493 gram substance gave 0.2625 gram AgCl (Carius).

	Theory for $(C_{12}H_{14}N_2O, HCl)_2.PtCl_4$.	Theory for Freer's $(C_{12}H_{16}N_2O, HCl)_2.PtCl_4$.	Found. I.	II.	III.	IV.	V.	VI.
C	35.44	31.70	35.50	35.43	...	...	...	...
H	3.69	2.91	4.02	4.03	...	...	...	...
N	6.89	7.39	...	...	6.78	7.07	...	...
Pt	23.92	25.63	...	...	...	...	23.61	...
Cl	26.10	28.14	...	...	...	...	...	25.91

The analyses agree so well with the formula $C_{12}H_{14}N_2O$ that there can be no doubt that in the above treatment acetacetic-

ether hydrazide has lost one molecule of water, forming phenyl-methyl ethoxypyrazol, as follows:

$$\begin{array}{l} CH_3.C\text{–}NH.N.C_6H_5 \\ \quad\quad\quad\

able, through a careful quantitative study of the decomposition of the silver salts of the halogen substitution-products of the fatty acids, to arrive at some general conclusion concerning the relative influence of the different halogens upon the decomposition, and to note the effect of the same halogen in different positions in the molecule, that is, to compare, for example, the rate of decomposition of α-brompropionate of silver with the same salt of the β-acid.

With this end in view, a quantitative study was made of the rate of decomposition of silver chloracetate in the following way and with the following results.

The Rate of Decomposition of Silver Chloracetate.

This salt was prepared by neutralising silver carbonate with an aqueous solution of chloracetic acid, and in this way was obtained in needle-shaped crystals which, on analysis, gave numbers closely agreeing with the theory. The salt thus obtained was ground to a fine powder and carefully preserved in a tightly-stoppered bottle in the dark, under which conditions it undergoes but little alteration.

The conditions of the experiments were these:

0.2015 gram of the silver salt, or the thousandth of one equivalent in grams, was heated in a sealed glass tube in a bath, the temperature of which was kept constant at 134°–135° C., with 9 cc. of water, that is, with 500 times the quantity necessary for the complete decomposition of the weight of salt taken.

The sealed tubes were made as nearly as possible of the same size, that is, about six inches long. The bath was made of thin copper after the manner of a Carius furnace, the outer jacket of the bath being connected with a return-condenser and partially filled with xylene, the boiling of which served to keep the inner tube of the bath at the above-named temperature. After each experiment the sealed tube was at once removed from the bath and allowed to cool for 10 minutes, when it was opened and the amount of undecomposed silver salt determined by means of a standard solution of ammonium sulphocyanide.

I am aware that the conditions of these experiments may be improved upon, and that such as they are they are by no means easy to realise exactly in every instance; and hence I do not give these results as the very best attainable and free from all error, but simply as a fairly close approximation to the truth. The table below gives the results obtained and shows the rate per minute of this decomposition.

TABLE I.—*Decomposition of the Silver Chloracetate at* 135° *C.*

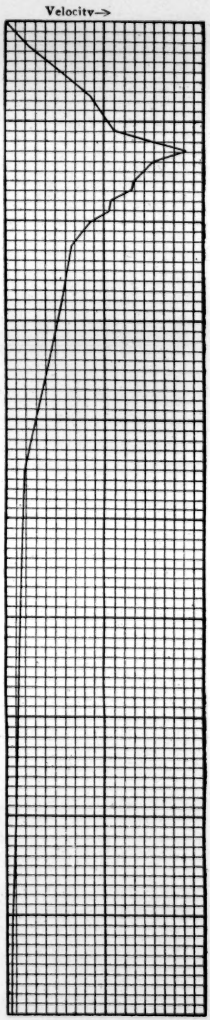
No. of Exp.	Substances taken.	Time in Bath at 135° C.	Percentage of Decomposition.	ϑ.
1	0.2015 gram silver chloracetate. 9 cc. of water.	5 min.	11.38	2.27
2	" "	10	48.92	8.54
3	" "	12	60.16	11.00
4	" "	13	67.36	18.07
5	" "	15	77.04	14.79
6	" "	16	80.	12.96
7	" "	17	82.60	13.
8	" "	18	84.45	10.63
9	" "	19	86.10	10.61
10	" "	20	87.3	8.62
11	" "	25	91.5	6.67
12	" "	30	94.	5.88
13	" "	60	97.24	1.8
14	" "	120	97.97	0.44

In the last column, marked ϑ, are placed the numbers showing the average rate of speed of the decomposition at the several intervals of time. These numbers were arrived at in this way.

In Exp. No. 1 there was decomposed 11.38 per cent. of salt; hence the average rate of decomposition during the first five minutes is 2.27 per cent. per minute. In Exp. No. 2 there must have been decomposed at the end of the first five minutes as much as in Exp. No. 1, that is, 11.38 per cent., and hence there was left undecomposed at the end of the first five minutes 88.62 per cent. of the silver salt. In Exp. 2, however, there was decomposed altogether 48.92 per cent., hence during the second five minutes of this experiment there was decomposed $48.92 - 11.38 = 37.54$ per cent. $88.62 : 37.54 :: 100 : x$. $x = 42.36$ per cent.

This number 42.36 represents then the actual percentage of salt decomposed during the last five minutes of the second experiment as calculated upon the amount of undecomposed substance at the beginning of the second five minutes, and this divided by five gives the number representing the average rate of decomposition during the second five minutes. The numbers in the column ϑ if used as ordinates in the construction of a curve in which the different increments of time are projected on the axis of abscissæ, give a curve which, in spite of several slight irregularities, illustrates very clearly the decomposition of silver chloracetate under the above-mentioned conditions.

A study of this curve shows that the decomposition rises rapidly to a maximum, which it reaches even before the temperature of the solution has reached the highest attainable temperature of the bath, which is 135°, and which is not reached until twenty minutes after the tube is introduced. Having reached this maximum, the decomposition proceeds more and more slowly in spite of the fact that at the end of twenty minutes the bath and its contents have reached the highest attainable temperature under the given conditions; and finally at the end of three hours the decomposition is almost if not entirely complete.



That the change which here takes place is not a reversible one was shown by heating together a known quantity of freshly precipitated silver chloride and an equivalent quantity of glycollic acid under such conditions as were stated above. Under these circumstances no trace of silver could be detected in the solution, thus proving conclusively that the reaction here studied is not, for these conditions at any rate, a reversible one. Such being the case it was thought that the retardation of the velocity of the change might nevertheless be caused by the presence and action of glycollic acid, which is one of the products of the decomposition, and in order to settle this point the following experiment was tried:

0.0760 gram of glycollic acid together with 0.2015 gram of silver chloracetate and 9 cc. of water were heated in the bath at 135° C. for 15 minutes. The amount of decomposition was found to be 75.27 per cent.

Comparing this with the amount of decomposition in Exp. 5, Table I, which is 77.04 per cent., it will be seen that the difference is exceedingly small, and hence the retarding effect of the glycollic acid is but slight, and can scarcely be looked upon as sufficient to account for the great retardation which the curve on p. 589 indicates. If this reaction then is a non-reversible one—and such it has been shown to be—and if the soluble product of the decomposition, glycollic acid, does not exert any very considerable influence on the velocity of the decomposition, then plainly we must look to the quantity of water present at the different times as furnishing the only explanation of the curve expressing the velocity of the change. As a matter of fact it has already been noticed by Beckurts and Otto that this decomposition is much accelerated when the quantity of water present is small; but as these observers were chiefly engaged in studying this kind of decomposition with reference to the end-products of the reaction, it is not surprising that they should have contented themselves with merely a passing notice of this remarkable fact.

In order to make sure that the retardation of the velocity of this decomposition was due largely to an ever-increasing quantity of water, a number of experiments were made, the conditions and results of which are given below in Table II.

TABLE II.—*Showing the Effect of Water on the Decomposition of Silver Chloracetate.*

No. of Exp.	Quantity of Silver Salt taken.	Quantity of Water.[1]	γ.	Time.	Temperature.	Amount of Decomposition.
1	0.2015 gram.	1.8 cc.	100	1 hour.	100° C.	69.1 per cent.
2	"	3.6	200	"	"	60.1
3	"	5.4	300	"	"	52.
4	"	7.2	400	"	"	47.28
5	"	9.	500	"	"	43.36
6	"	18.	1000	"	"	31.13
7	"	27.	1500	"	"	23.70
8	"	36.	2000	"	"	21.
9	"	54.	3000	"	"	17.02
10	"	90.	5000	"	"	12.48
11	"	180.	10,000	"	"	8.30
12	"	360.	20,000	"	"	5.95
13	"	540.	30,000	"	"	3.10

[1] The quantity of water which actually enters into reaction with the quantity of silver salt used in each of these experiments is 0.0180 gram.

In this table, in the column marked γ, are given numbers which represent the several quantities of water taken, in terms of the quantity necessary to secure the complete decomposition of the quantity of salt used as unity. In Exp. 5, for example, 500 in column γ signifies that the quantity of water actually employed, that is 9 cc., is 500 times the quantity necessary completely to decompose 0.2015 gram of the silver salt, $500 \times 0.0180 = 9$.

The results obtained in this last series of experiments show beyond a doubt that the quantity of water exerts a very marked and regular influence upon the course of this reaction; and they likewise enable one to account for the results obtained in the first series of experiments, and to interpret the curve on p. 589 as follows: When a tube containing 0.2015 gram of silver chloracetate and 9 cc. of water is introduced into the bath whose temperature is 134°–135° C., we may safely assume that the decomposition begins almost immediately, for the reason that the temperature of the bath under these circumstances never falls below 100° C., and at this temperature the decomposition proceeds fairly rapidly. At the beginning of the experiment we have 500 times the quantity of water necessary to insure the complete decomposition of the salt; and, as the temperature of the bath gradually rises, the decomposition proceeds with increasing velocity. Nevertheless, as more and more and more of the salt is decomposed, so larger and larger (proportionately) becomes the excess of water, so that after the maximum is reached, the curve, notwithstanding the fact that the temperature is constantly becoming higher, begins a gradual descent, because of the retarding effect of the increasing quantities of water. In order to determine more fully the influence of the glycollic acid upon the course of the reaction, the following experiments were tried, the results and conditions of which are indicated in Table III. In column VI of this table are also given, for the sake of comparison, the results before indicated in Table II.

It will be seen from this that the general effect of the glycollic acid is one of retardation; but a careful study of any of the results thus obtained—as was the case also with that obtained at 135° C. and cited above—reveals the fact that the retarding influence thus exerted is by no means sufficient to account for the great retardation experienced on dilution with water.

TABLE III.—*Showing the Effect of Glycollic Acid on the Decomposition of Silver Chloracetate.*

I.	II.	III.	IV.	V.	VI.	VII.	VIII.
No. of Experiment.	Silver Salt Taken.	Quantity of Water.	Quantity of Glycollic Acid.	Percentage Amount of Decomposition.	Taken from Table II.	Retardation by Glycollic Acid.	Remarks.
1	0.2015 gr.	1.8 cc.	0.076 gr.	62.2	69.1	6.9	Time = 1 hr.
2	"	3.6	"	49.34	60.1	10.76	Temp. = 100° C.
3	"	5.4	"	43.95	52.	8.05	
4	"	7.2	"	41.86	47.28	5.42	
5	"	9.	"	38.9	43.36	4.46	
6	"	18.	"	27.25	31.13	3.88	
7	"	27.	"	20.82	23.70	2.88	
8	"	36.	"	18.61	21.00	2.39	
9	"	54.	"	12.55	17.02	4.47	
10	"	90.	"	8.33	12.48	3.65	
11	"	180.	"	5.44	8 30	2.86	
12	"	360.	"	3.35	5.95	2.60	
13	"	540.	"	0.89	3.10	2.21	

For the sake of illustration, the consideration of two of the experiments upon this point will suffice: In Exp. 1, Table III, we find that when 0.2015 gram of silver chloracetate is heated with 1.8 cc. of water at 100° C. for one hour, 69.1 per cent. of the salt is decomposed; whereas, in Exp. 10, the conditions being the same except that here 90 cc. of water are used, we find only 12.48 per cent. of the salt decomposed. In no case now does the full equivalent of glycollic acid produce a retardation greater than 10.76 per cent. (see column VII, Table III), the average being much less. Inasmuch as the glycollic acid formed in Exp. 10 as the result of the decomposition is only about one-eighth of the quantity used in the experiments tried for the purpose of discovering its effect, it follows that the retardation in this particular case due to the glycollic acid could hardly be greater than 2 per cent. Owing to the difference in the quantities of water employed in these two experiments 1 and 10, however, we find a retardation of 56.62 per cent., or 54.62 per cent. above that which could have been brought about by the glycollic acid. Again, a comparison of the results of Experiments 1 and 10 in columns V and VI, Table III—the conditions of which were exactly the same except that in those of column V one equivalent, 0.076 gram of glycollic acid was introduced—shows the retardation produced by the same quantity of

water to be in each case nearly the same, being in the one 53.37 per cent. and in the other 56.62 per cent. In fact it may be said that the results given in these two columns V and VI illustrate equally well, and in most instances to almost exactly the same degree, the retarding effect of water upon the course of the reaction.

The decomposition of silver chloracetate in the presence of water into glycollic acid and silver chloride is a clean one and, so far as I have been able to discover, unattended by the formation of secondary products of any kind; and, furthermore, the silver chloride exerts no influence upon the rate of the decomposition, as is shown by the following experiments:

I. 0.2015 gram silver chloracetate and 0.1435 gram silver chloride—an equivalent quantity—heated with 36 cc. water at 100° C. for 1 hour, gave 21.1 per cent. decomposed.

II. Conditions, substances and quantities same as I gave 20.8 per cent. decomposed.

Comparing these results with that of Experiment 8, Table II, which is 21 per cent., we see that the silver chloride exerts no influence upon the rapidity of the change.

The retarding influence of water on the velocity of the decomposition, and it being, moreover, the substance which *apparently* produces the change and which certainly does enter into reaction with the other substances involved, shows that this reaction, in spite of its simplicity, is anomalous.

For these facts, remarkable as they are, no explanation has ever been offered, so far as I know. As stated above, Beckurts and Otto were the first to note that the decomposition of silver chloracetate proceeds more rapidly in a small than in a large quantity of water; they offer no explanation of the fact, however, and in all of their papers upon this and closely related subjects which have come under my notice—and there are several—nothing has been found by way of explanation or of theoretical significance beyond a brief statement concerning certain experiments with dichloracetic acid, the import of which is that the rapidity of the decomposition of the silver salt of this acid depends upon a destruction of the equilibrium due to the insolubility of silver chloride, and not *solely* upon the affinity of the metal for the halogen.[1]

While this is doubtless true, and applicable perhaps to the

[1] Ber. d. chem. Ges. **14**, 582.

decomposition of the silver salt of monochloracetic acid as well, this explanation is not sufficiently far-reaching, and the only explanation of the facts above stated which seems at all plausible and worthy of consideration is that furnished by Ostwald's studies on the affinity of acids. According to this distinguished investigator, the strength or, better perhaps, the affinity of any acid depends upon the extent to which it is dissociated into its ions, and all acids would have equal affinities if fully dissociated.[1] He has proved experimentally that weak acids become rapidly stronger as the dilution increases. and believes the cause of this to be that such acids are but little dissociated in their concentrated state or at moderate dilution: dissociation, increasing with the dilution, greatly increases the affinity of these acids. Strong acids, on the other hand, like hydrochloric, which are already tolerably far dissociated in their concentrated state, and at moderate dilutions, do not gain much in strength by further dilution. For the sake of clearness regarding the effect of dilution upon the relative affinities of strong and weak acids a few selections have been made from Ostwald's tables and are given below in Table IV.

TABLE IV.—*Relative Affinities of Acids at Different Dilutions.*

	Strength of Solution.		
Name of Acid.	Normal.	$\frac{1}{5}$ Normal.	$\frac{1}{10}$ Normal.
Nitric,	1.00	1.00	1.00
Hydrochloric,	.90	.98	.979
Chloric,	.932	...	.998
Acetic,	.0094	.0123	.0735
Monochloracetic,	.046	.070	.213
Dichloracetic,	.165	.33	.488

It will be seen that further dilution does not increase the affinity of strong acids which are moderately dilute. Hydrochloric acid, for example, is increased only .08 in passing from a normal to a $\frac{1}{5}$-normal state of dilution, or becomes only about one-eleventh stronger, and having reached this strength it is not increased at all in passing to a $\frac{1}{10}$-normal state of dilution. Monochloracetic acid, on the other hand, which is a weak acid, rapidly increases in strength as it is diluted, being in $\frac{1}{10}$-normal solution about five times as strong as it is in the normal. In the light of

[1] Outlines of General Chemistry. By W. Ostwald. Translated by James Walker. p. 361.

this theory of affinity, then, the decomposition of silver chloracetate in the presence of water, and the retardation of the change produced by the excess of water, may be explained in this way: we have in the compound, silver chloracetate, $CH_2Cl.COOAg$, two acids, one hydrochloric, the other chloracetic, exerting their affinity on one metal, silver.

This may be expressed graphically by the aid of these two formulas:

$$\begin{matrix} Cl \longrightarrow Ag \\ | \\ CH_2COO \end{matrix} \quad \text{and} \quad (CH_2ClCOO) \rightarrow Ag.$$

The one of these two acids, hydrochloric, is one of the strongest acids known, and, according to Ostwald, its strength will not be greatly increased by dilution with water, in fact it will not be increased at all appreciably after the state of moderate dilution is passed.

It will be seen from the above table, for example, after converting the numbers there given into whole numbers by multiplying by 100, that the relative affinity of this acid is 90 in normal solutions, 98 in $\frac{1}{3}$-normal, and essentially the same in $\frac{1}{10}$-normal, that is 97.9. Chloracetic, on the other hand, is a weak acid, capable, however, of showing a greater and greater affinity as it is diluted. In normal solutions, for example, its relative affinity is 4.6; in $\frac{1}{2}$-normal it is 7, or nearly twice as strong; and in $\frac{1}{10}$-normal it is 21.3, or nearly 5 times as strong as it is in the normal state of dilution. Hence we may imagine it to become through dilution as fully dissociated, and hence as strong, as hydrochloric acid itself. The facts are, with reference to the decomposition of chloracetate of silver, a salt in which both of these acids—the one potentially, the other actually—are exerting their affinity upon the silver, that the decomposition of this substance, in the dry state, and in that in which both of these acids, so to speak, are in a highly concentrated form, and therefore the one very strong and the other very weak, is one of an explosive kind, that is, the affinity of the hydrochloric acid so far exceeds that of the monochloracetic acid as to break down the molecule, the decomposition taking place as rapidly as possible in the sense of this equation:

$$CH_2Cl.COOAg = AgCl + CH_2\begin{matrix} CO \\ / \quad \backslash \\ \text{——} \quad O \end{matrix}.$$

When the silver chloracetate is mixed with a small quantity of water—as it is in Exp. 1, Table II, for example, where it is in nearly ½-normal solution—the affinity of the hydrochloric acid probably becomes as great as 95, or one-eighteenth stronger; on the other hand, the affinity of the chloracetic acid probably becomes 6, or about one-fourth stronger. Although the hydrochloric acid is still stronger and much stronger, nevertheless the balance of the increase in strength is in favor of the chloracetic acid, and hence the decomposition, while taking place rapidly, does not begin to compare in rapidity with that which takes place in the dry state, or with just a drop or two of water: in other words, the presence of even a comparatively small quantity of water, by virtue of its more greatly increasing the strength of the chloracetic than of the hydrochloric, has retarded the action to a considerable extent.

In the light of this hypothesis, the results obtained in the several series of experiments are no longer difficult to explain: the regular retardation of the velocity of the change as shown in Table II is accounted for on the assumption that the affinity of the hydrochloric acid soon becomes a constant force, and one which is sufficient to disintegrate the molecule in question; and that opposing this constant force is another, that of the affinity of chloracetic acid, which, while much weaker than the former at the start, rapidly increases in strength in consequence of dilution, until at last it becomes almost, if not quite, powerful enough to hold it, the former, completely in check, and chemical equilibrium is the result.

I am not unaware that there may be, and doubtless are, other elements in this problem than those which are here discussed; nor unmindful that the experimental difficulties in the way of an ultimate solution of it are many; nevertheless this hypothesis opens up an interesting field of inquiry and research, and is important not only in its immediate application, but also in its more remote, though direct, bearing upon Ostwald's theory of affinity as well.

Work is now in progress, the purpose of which is to test this hypothesis with regard to the decomposition of other salts of this acid, and likewise the various salts of bromacetic and other closely related acids. It may be said in this connection that already sufficient experimental data are at hand concerning the decomposition of potassium chloracetate to indicate that its decomposition in water, like that of the corresponding silver salt above discussed, suffers a gradual retardation with increasing quantities of the solvent.

This is all the more interesting in consequence of the fact that potassium chloride is so easily soluble in water, and hence does not disturb the reaction by being removed from the field in consequence of precipitation, as is the case with the silver salt. The effect of the glycollic acid upon the change will also, in this case, be exhaustively studied in the hope of being able to define more exactly the influence which it exerts upon the speed of such decompositions; and in consequence of the greater possibilities presented by this potassium salt, it is hoped to arrive ultimately at a full and complete explanation of the mechanism of this interesting phase of chemical action.

In conclusion I desire to thank Mr. B. C. Keiser for his excellent assistance in the experimental part of this investigation.

A. AND M. COLLEGE OF KENTUCKY, LEXINGTON, *June*, 1892.

ON THE PREPARATION AND CONSTITUTION OF PARA-ANTHRACENE.

BY C. E. LINEBARGER.

The action of sunlight upon solutions of anthracene was first observed and studied by Fritzsche,[1] who describes the geometrical form of the crystals thus obtained from alcoholic and benzene ("Steinkohlenöl") solutions. Graebe and Liebermann,[2] in their work on alizarine, also prepared the substance, without making, however, any considerable study of it. Schmidt,[3] in investigating the action of nitric acid on anthracene, also occupied himself with a study of the behavior of para-anthracene towards nitric acid. Recently Elbs[4] has determined the solubility of para-anthracene in various solvents, and cryoscopically its molecular mass. This he found to be double that of anthracene, viz. $(C_{14}H_{10})_2$.

The method generally employed in the preparation of para-anthracene consists in exposing a solution of anthracene in benzene[5] to the direct action of the sun's rays. The almost insoluble

[1] J. prakt. Chem. **101**, 337.
[2] Ann. Chem. (Liebig) Supl. **7**, 264.
[3] J. prakt. Chem. [2] **9**, 248.
[4] Ibid. [2] **44**, 467.
[5] Fritzsche (*loc. cit.*) employed alcohol also.

para-anthracene separates out in the form of minute white crystals which adhere so firmly to the sides of the vessel in which the solution is contained that they cannot be entirely removed by mechanical means. This crystalline crust shuts out much light and thus retards the speed of transformation. Thinking that this phenomenon might not occur if other solvents were used, I prepared saturated solutions of anthracene[1] in dry chloroform and in dry carbon bisulphide.[2]

After an exposure of a few days, white glancing crystals appeared floating about on the surface of the chloroformic solution, which had turned almost black. When no more crystals seemed to separate out, most of the chloroform was distilled off, and the dark-colored residue filtered and washed with cold acetic ether. A perfectly white crystalline mass was thus obtained, melting well at 243°, and is therefore para-anthracene. No difficulty was experienced from the crystals sticking to the sides of the vessel, as in the case of benzene solution.

The solution of anthracene in carbon bisulphide also turned black after a few days of exposure, but no separation of crystals took place. The carbon bisulphide was distilled off, and the black residue, in which could be seen many small glistening tablets similar to those of anthracene, was washed with cold acetic ether until the washings were not colored. The resulting grayish-white mass melted at 208°–210°, *i. e.* the melting-point of anthracene. Para-anthracene is, then, not formed from anthracene in carbon-bisulphide solution. This shows that the solvent has a certain effect upon the formation of para-anthracene from anthracene, and that the transformation does not take place in every solvent employed.

The experiments of Beckmann[3] show that the nature of the solvent has considerable influence upon the size of the molecules of the dissolved substance.

[1] The pure hydrocarbon was prepared as follows: Ordinary green anthracene, containing about 60 per cent. of the pure hydrocarbon, was heated with reflux condenser for 24 hours with about twice its volume of acetic ether. The dark-colored solution was filtered off, and the residue washed with cold acetic ether until the washings passed through colorless. The light green product was subjected to sublimation, the process being accelerated by passing a rapid current of air over the melted anthracene contained in a retort. By a second sublimation, quite pure anthracene was obtained.

[2] Carbon bisulphide was hardly a suitable solvent, as it is itself decomposed by sunlight. Yet this decomposition takes place so slowly that it was at first thought that that circumstance would have but little effect upon the transformation of anthracene into para-anthracene.

[3] Ztschr. phys. Chem. **6**, 437.

Compounds dissolved in the alcohols, phenols, ethers, etc., generally have a normal molecular mass, while when dissolved in benzene, carbon bisulphide, chloroform, ethylene bromide, etc., they have a tendency to form more complex, especially double, molecules. It is not admissible to give as the reason of the non-transformation of anthracene into para-anthracene in carbon bisulphide solution, that the anthracene exists in solution as a double molecule, although such a view of the case is quite in accordance with a hypothesis (to be given below) which I have made in regard to the constitution of para-anthracene; for Beckmann[1] has shown that anthracene has a normal molecular mass when dissolved in carbon bisulphide. The matter was deemed of sufficient importance to warrant the investigation of the behavior of anthracene dissolved in a variety of solvents towards sunlight. As anthracene is not very soluble in most liquids, in order to reduce the quantity of solvent the samples were prepared as follows: Somewhat more anthracene than could be dissolved was added to each of the solvents; as soon as the anthracene changed into para-anthracene, a fresh portion of the former hydrocarbon entered into solution. Thus with a small quantity of the solvent a comparatively large quantity of anthracene could be transformed into para-anthracene.

The solutions were exposed about two months (summer of 1892). The precipitates of para-anthracene were then filtered off, washed with a little of the pure solvent, dried, and their physical properties noted. These are here given as briefly as possible, the examination of the crystals being done, of course, with the aid of a microscope.

Anthracene[2] dissolved in—

1. *Benzene*, colorless solution, six-sided prisms, m. p. 243°–244°.

2. *Toluene*, colorless solution, rhombic tablets, m. p. 244°.

3. *Xylene* (Kahlbaum's *puriss.*), colorless solution, rhombic tablets, exceptionally numerous and well formed, m. p. 244°.

4. *Ethyl benzene*, colorless solution, comparatively large elongated hexagonal plates, m. p. 244°.

[1] *Loc. cit.*, p. 447.

[2] The anthracene employed in these experiments was prepared from anthraquinone by reduction with ammonia and zinc dust, and had been crystallised from alcohol until it possessed the bluish fluorescence characteristic of the pure substance.

5. *Alcohol* (99 per cent.), light-yellow solution, well defined rhombs, with an occasional hexagonal plate, m. p. 244°.

6. *Acetic acid* (glacial), light-yellow solution, poorly crystallised, only a few well defined rhombs present, m. p. 243°–245°.

7. *Ethyl benzoate*, light-yellow solution, six-sided plates, m. p. 244°.

8. *Benzene bromide*, solution yellow, hexagons few in number, m. p. 244°.

9. *Chloroform*, solution quite black, rhombic plates, not well crystallised, m. p. 244°.

10. *Ethylene bromide*, solution almost black, grayish-white plates similar to those of anthracene, green coloration with concentrated sulphuric acid, m. p. 210°–212°.

11. *Carbon bisulphide*, black solution, indefinitely crystallised, m. p. 207°–211°, green coloration with concentrated sulphuric acid.

The above experiments show that in the transformation of anthracene into para-anthracene, the solvent not only has influence upon the product, but is itself changed in some instances. The hydrocarbons of the benzene series remain unaltered, but all the solvents containing oxygen, although of various chemical constitutions, take on a light-yellow color, which indicates that some chemical change has probably taken place. This color does not deepen, even if the solution be exposed for a long time (four months) to sunlight, when once all the anthracene has been transformed into para-anthracene, showing that the change of color takes place only during the actual transformation of the anthracene into its polymer. The shade of yellow of all the solutions was about the same.

One other solvent besides carbon bisulphide was found not to permit of the transformation of the anthracene, viz. ethylene bromide. This is undoubtedly due to the fact that ethylene bromide, when exposed to the sun's rays, is itself decomposed into a brown, amorphous substance, adhering firmly to the glass, while the liquid assumes a dark-brown color. The decomposition does not proceed so rapidly as in the case of carbon bisulphide.

The non-transformation of anthracene into para-anthracene in carbon bisulphide and in ethylene bromide solution cannot be owing to the blackening of the solution and consequent exclusion of the light, for in chloroform solution, which turns black as

rapidly as carbon bisulphide, the change takes place quickly; nor can it be owing to the presence of the halogens (bromine) in the molecule of the solvent, for the transformation is effected in benzene bromide with but slight change of color. Neither chloroform nor benzene bromide is decomposed by the direct action of the sun's rays; hence the non-transformation of anthracene into para-anthracene when dissolved in carbon bisulphide or ethylene bromide must be on account of the decompositions of the solvents. What the products of these decompositions are it is difficult to say, as they are black, amorphous masses easily undergoing alteration.

Of all the solvents used, xylene proved itself the best adapted for the preparation of para-anthracene. If a bottle nearly full of xylene to which nearly the equal weight of anthracene has been added, be exposed to sunlight, in a little while (depending of course upon the amount of sunshine) all the solid hydrocarbon will have been changed into its polymer, which can easily be separated from the last traces of anthracene by washing with hot benzene.

As stated above, para-anthracene has a molecular mass double that of anthracene, $C_{14}H_{10}$. The question arises, in what way do two molecules of anthracene unite to form one molecule of para-anthracene? So far as I know, no answer has been given to the question; accordingly the following is offered.

It is pretty well established that in anthracene, the so-called γ-carbon atoms have one bond in common, *i. e.* anthracene may be regarded as ethane in which four of the hydrogen atoms are replaced by two bivalent radicles, viz. phenylene; or, what amounts to the same thing, anthracene consists of three benzene rings, of which the middle one has two of its carbon atoms, in the para-position, bound directly together. This double linkage of the γ-carbon is quite loose, as shown by the ready oxidation of anthracene into anthraquinone, and the formation of addition-products with chlorine and bromine at low temperatures. It might be now that by the action of the sun's rays these double bonds would be set free in some way. If, then, a molecule of anthracene with its double bonds thus loosed should come within the sphere of action of another molecule in a similar condition, the free bonds of the one molecule might unite with the free bonds of the other, doubling up and forming a new molecule stable so far as the

action of light is concerned. In direct sunlight, the equilibrium of the anthracene molecule is labile, while that of the para-anthracene is stable. On the other hand, under the influence of heat the para-anthracene molecule is in labile, and the anthracene in stable equilibrium, for at 244° the double molecule decomposes into two simple ones—para-anthracene changes into anthracene.

There is then in para-anthracene a ring of four carbon atoms, occupying with respect to the anthracene molecule the γ-position. No addition- or substitution-products of this ring have as yet been obtained, although it might be possible by means of the Friedel-Crafts reaction in carbon bisulphide solution to effect the substitution of bromine for hydrogen.

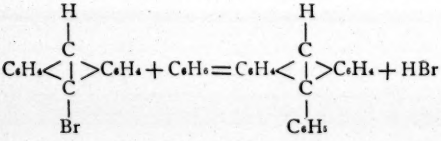
It is my intention to carry out some experiments with that end in view, as well as to determine what kind of light is most effective in transforming anthracene into para-anthracene.

CHICAGO, *September*, 1892.

ON THE APPLICATION OF THE FRIEDEL-CRAFTS REACTION TO SYNTHESES IN THE ANTHRACENE SERIES.

BY C. E. LINEBARGER.

This paper contains an account of some experiments carried out with a view to effecting syntheses in the anthracene series by means of the action of aluminium chloride upon the γ-halogen (Cl, Br) substitution-products of anthracene in benzene solution. Although but negative results were obtained, they are of sufficient importance to merit publication and may serve to prevent others from working over the same ground. Theoretically, it is not impossible to obtain the phenylanthracenes by the application of the Friedel-Crafts reaction, according to the equations:



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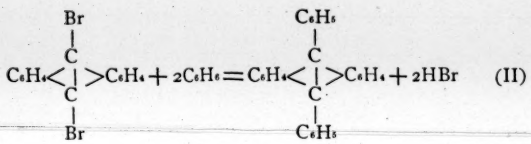


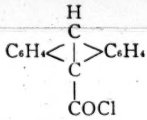
The following is a statement of my attempts to realise the above equations given as briefly as is consistent with clearness.

20 grams of powdered aluminium chloride were added to a solution of 20 grams of anthracene monobromide in 150 grams of benzene. At the ordinary temperature no reaction took place, but at about 50° C. hydrobromine acid commenced to pass off, and the reaction proceeded quite briskly at 60° to 70° C. When the evolution of gas slackened a little, I heated to boiling for a few minutes. The amount of hydrobromic acid given off corresponded to the theoretical quantity calculated according to equation (I). The product of the reaction, when cool, was treated with water in the usual way. The benzene solution was then separated from the water, dried over calcium chloride and distilled without a thermometer. After the benzene had been driven over, a reddish-brown distillate was obtained with high melting-point. This distillate was treated with hot alcohol and the solution filtered. Small, glistening scales just like those of anthracene separated out from the solution when it became cold. These crystals melted at 207° to 210° C. and gave the characteristic green color with concentrated sulphuric acid. About 5 grams of this crystalline product, which was undoubtedly anthracene, were obtained. Not a trace of phenylanthracene could be found. I repeated this experiment several times with varying quantities of aluminium chloride, benzene and monobromanthracene, but always obtained about the same results.

I went through similar experiments with dibromanthracene, benzene and chloride of aluminium, but never succeeded in obtaining any crystallisable compounds other than anthracene, although a considerable amount of resin was formed, which was very soluble in benzene. The reaction did not begin below the boiling-point of benzene, and then proceeded rapidly, the theoretical amount of hydrobromic acid, calculated according to equation (II), being given off.

In one experiment I employed dichloranthracene, but obtained the same products as with anthracene dibromide.

Graebe[1] has found that by heating anthracene and phosgene together in a sealed tube, a compound having the formula



is formed. I tried to obtain the same substance by letting chloride of aluminium react with anthracene and phosgene dissolved in carbon bisulphide—about the only solvent not itself attacked by the metallic chloride. So I passed a stream of phosgene gas into a solution of anthracene (prepared by reduction of anthraquinone) in carbon bisulphide to which some powdered aluminium chloride had been added. When the solution was saturated with the gas,[2] I gradually heated it to boiling, without, however, a reaction taking place. No hydrochloric acid was given off and the whole of the anthracene was recovered from the solution.

CHICAGO, *September*, 1892.

THE DISSOCIATION OF SALTS INTO THEIR IONS BY WATER OF CRYSTALLISATION.

BY C. E. LINEBARGER.

It is well known that many salts which form crystalline compounds with water assume different colors according to whether they be in the anhydrous or hydrated state; also that the intensity of color varies according to the proportion of water present. Anhydrous copper sulphate, for instance, is white, while its several hydrates are blue. Aqueous solutions of copper sulphate are blue also. According to the electro-chemical theory of Arrhenius, this blue color of the solutions of copper sulphate is due to the pres-

[1] Graebe and Liebermann: Ber. d. chem. Ges. **2**, 678.

[2] Phosgene is very soluble in carbon bisulphide.

ence of blue copper ions, for the SO_4 ions are colorless as well as the anhydrous molecule. Similarly all solutions of salts containing but one colored ion owe their color to that ion. In the case of salts, the anhydrous molecule as well as each of the ions of which are colored, the color of the solution is the resultant of the colors of the free ions and the undecomposed molecule. Now, if it be admitted that the color of a solution of a salt be due to the presence of colored ions, it must be admitted also that the color of the same salt crystallised with water of crystallisation is due to the presence of free ions, or, in other words, *the water of crystallisation has the power of dissociating crystallised salts into their ions.* Thus, in the example chosen, copper sulphate containing blue copper ions has a blue color if water of crystallisation or of solution be present; otherwise it is white. In the case of colorless salts composed of colorless ions, it follows from analogy that the above statement obtains also.

Considerations of a somewhat different nature lead also to the conclusion that salts crystallising with water may decompose into their ions. The hard-and-fast line that was once supposed to separate the liquid from the solid state of aggregation does not in reality exist. Solids have the faculty of forming solid solutions,[1] which diffuse, exert an osmotic pressure, and, what is of vital importance for the question at issue, are in many cases conductors of electricity of the second class. The fact of electrolysis renders necessary the assumption of electrical dissociation. Vapors in many cases are true electrolytes; innumerable solutions have been found to be such, as well as many salts in the vicinity of their melting-points. So far as I know, no experiments have been carried out to test the electrical conductivity of salts containing water of crystallisation. The degree of dissociation of salts in solution is generally determined from the osmotic pressure or from the electrical conductivity. The methods in use may not be exactly suited to solids, but undoubtedly others will be found. Thus the experiments of Graetz[2] and Poincaré[3] show that at the melting-points of salts there is no abrupt change in the electrical dissociation or in the friction of the ions. Accordingly, it may be expected that at the melting-points of hydrated salts the electrical conductivity is the same in both liquid and solid state; knowledge

[1] J. H. Van't Hoff: Zeitschr. f. phys. Ch. **5**, 322. [2] Wiedemann Ann. **40**, 18.
[3] Ann. de chim. et phys. (6) **21**, 289.

may thus be gotten of the degree of dissociation in the solid state. The investigation of the magnetic rotation and the refractive index will also contribute to our knowledge of the subject.

The most general conclusion drawn from the assumption of free ions in solution is that the properties of solution can be represented as binary sums of the properties of both ions. This conclusion must obtain also for salts crystallised with water. But as the conclusion in regard to solutions is strictly true only for infinite dilutions, we may expect to find many seeming exceptions in the case of crystallised salts where the concentration is very great. Still, from the data at hand, I think that it may be stated as a general law that *salts crystallising with water of crystallisation are more or less decomposed into their ions.*

CHICAGO, *September* 20, 1892.

THE IODOMERCURATES OF ORGANIC BASES.

BY ALBERT B. PRESCOTT.[1]

An iodide of mercury and an alkaloid is not merely a double halide of two unlike bases. In character it is not at all a mean between mercuric iodide on the one hand and an iodine compound of the alkaloid on the other. So far as it can be compared with any iodine compounds of alkaloids, it resembles the unions of free iodine more than the salts of hydriodic acid. But the element mercury joins its influence with that of the iodine to constitute an iodomercuric acid group of decided affinity for certain bases. These certain bases are those typified by pyridine and by the quinolines—tertiary bases carried in closed carbon chains.

An iodomercurate is evidently a strongly individualised compound, as, in a degree, a ferrocyanide differs widely from a mean between the simple cyanides of the two metals it contains. But the iodomercuric-acid group exhibits its character only with the nitrogenous aromatic bases. Iodomercurates are to be compared with chloroplatinates, and, in a degree, with chloraurates, hardly with

[1] Read at the Rochester Meeting of the American Association for the Advancement of Science, August, 1892.

alkaloidal halogen compounds of cadmium, or bismuth, though all these make some definite combinations with alkaloids.

As a free element iodine effects a class of combinations with alkaloids generally, such as bromine and chlorine fail to effect. Apparently iodine atoms, more readily than those of bromine or chlorine, link together in a polyvalent group binding two other groups to each other. Again two atoms or three atoms of iodine seem to take the place of a monovalent group, as hydroxyl or amidogen would. All these considerations require a systematic study of the iodine compounds of the alkaloids, and bear upon the structure of iodomercurates.

When there shall be a surer understanding of the linkings of nitrogen in such compounds as ferrocyanides, and of the linkings of pairs of halogen atoms, it will become profitable to compare the part of iodine in the metallo-iodides with that of cyanogen in metallo-cyanides.

The iodomercurates are chiefly known in literature as precipitated products of potassium-mercuric iodide. A solution of this double halide was announced as a reagent for alkaloids in 1827,[1] in 1829,[2] and in 1830.[3] In 1846, Planta-Reichenau[4] reported the use of the tenth-normal solution of the reagent, substantially as published by Mayer in 1861.[5] Just before this, Groves[6] reported a research "On some Compounds of Iodide and Bromide of Mercury with the Alkaloids." The volumetric factor of the decinormal solution has been the subject of work by Dragendorff,[7] and later, in this country, by Lyons and by Snow.[8]

Following mainly the purpose of Groves, with difference of method, the writer several years ago presented some determinations of the iodomercurates of several vegetable alkaloids.[9] These did not agree very well in all respects with the figures of Groves, though somewhat more consonant with rational formulæ. To leave least possible doubt of the results, full elementary analyses

[1] P. Boullay, 1827: Ann. der Phys. Pogg. **87**, 99; Ann. chim. phys. [2] **34**, 345.

[2] P. A. von Bonsdorff, 1829: Ann. der Phys. Pogg. **93**, 243.

[3] F. L. Winckler, 1830: "Buchner's Repertorium," **35**, 57.

[4] A. von Planta-Reichenau, 1846: "Das Verhalten der Alkaloide gegen Reagentien"—a dissertation at Heidelberg.

[5] F. F. Mayer, 1862: Am. J. Phar. **35**, 20; Chem. News **7**, 159; **8**, 177, 189.

[6] Thomas B. Groves, 1858: Chem. Soc. **11**, 97; Phar. J. Trans. **18**, 181.

[7] G. Dragendorff, 1874: "Werthbestimmung starkwirkender Droguen."

[8] A. B. Lyons, 1886: Am. J. Phar. **58**, 579. H. W. Snow, 1888: *Ibid.* **60**, 487.

[9] A. B. Prescott, 1880: This Journal **2**, 294.

of iodomercurates have been made by Mr. Lyman F. Kebler, and to report these is the chief object of this communication.

The precipitates were those of quinine, of pyridine, and of quinoline. The quinine was purified by Mr. Kebler, the sulphate being recrystallised from hot water three times, the crystals being washed each time with cold water. This salt was precipitated by sodium hydroxide, and the washed precipitate, brought to constant weight at 120° C., taken as material for the work.

Observers have so generally found, with different alkaloids, a variability in the volumetric value of Mayer's reagent, as well as a point of unstable equilibrium in its use, that it seemed desirable to determine the elementary composition of an iodomercurate precipitated in several different degrees of concentration. Therefore the purified quinine, as a sulphate, was precipitated from four solutions, in 25, 50, 75 and 100 parts of water respectively to one part absolute quinine. An excess of the reagent was avoided in the precipitation. The precipitates, washed with water, were dried to constant weight at 60° C., for the elementary analysis.

The method of elementary analysis, in view of the presence of mercury and iodine, was a matter of experimentation with Mr. Kebler, who settled upon a procedure as follows: In the combustion for carbon and hydrogen, two U-shaped "chloride of calcium tubes" loosely filled with glass wool, and otherwise empty, were interposed between the combustion tube and the chloride of calcium. The first U-tube receives mercuric iodide and mercury during the combustion, the second one does not. To recover all the water of combustion from the glass wool, the dry air current is continued through the apparatus, with the combustion tube hot, drawing the current with an aspirator, or with a water-pump provided with a large regulator, for three to five hours. Heat may not be applied to the tubes interposed for mercury, nor can the glass wool be substituted by asbestos.

The nitrogen was estimated in the direct method, without interference by the mercuric iodide. For the mercury, a weighed portion of the dry iodomercurate was dissolved in alcohol acidulated with sulphuric acid, and the solution treated with gaseous hydrogen sulphide for full precipitation. For the iodine, a weighed portion of the dry iodomercurate was dissolved in alcohol acidulated with nitric acid, and a saturated solution of silver nitrate added to complete precipitation. For both the precipitates so

obtained, the mercury sulphide and the silver iodide, carbon tubes (such as are used in iron analysis) were prepared by placing a spiral of platinum in the bottom of each tube and filling over for about two centimeters in depth with finely-shredded asbestos suspended in water, then drying to a weight constant at 175° C. The two precipitates, for mercury and for iodine, were filled into the two tubes prepared, exhausted of liquid and washed with a filter-pump, and dried at 175° C., to a constant weight, when the weight of the prepared tubes was subtracted. This procedure was adopted by Mr. Kebler after trials of several other ways. Owing to the unusual features of the analysis, the results were obtained by Mr. Kebler in quadruplicate for each of the four quinine precipitates, and were as follows:

	I.		II.		III.		IV.	
C	20.92 21.10 21.12 20.92	21.01	20.86 20.69 20.43 20.91	20.87	20.82 20.66 20.78 20.71	20.74	20.96 20.67 20.72 20.70	20.72
H	2.27 2.19 2.26 2.24	2.24	2.49 2.25 2.21 2.33	2.32	2.41 2.73 2.29 2.37	2.45	2.21 2.73 2.43 2.34	2.42
N	2.53 2.41 2.35 2.51	2.45	2.33 2.31 2.34 2.30	2.32	2.51 2.32 2.26 2.42	2.38	2.89 2.57 2.36 2.24	2.51
Hg	23.13 23.16 23.21 23.12	23.20	23.15 23.12 23.56 22.96	23.28	24.03 22.27 23.29 22.81	23.10	23.20 23.36 23.66 23.49	23.42
I	47.59 47.51 47.68 47.70	47.62	47.22 47.60 47.59 47.39	47.45	47.41 47.56 47.25 47.35	47.39	47.21 47.36 47.40 47.53	47.42
O	3.56 3.63 3.35 3.51	3.51	3.95 4.03 3.87 4.11	3.99	2.82 4.46 3.74 4.34	3.84	3.53 3.33 3.43 3.51	3.41

It appears, therefore, that the composition of the precipitates is not varied by the dilution of the solution in which precipitation is effected. And all the results are in quite good agreement, giving

redundant proof of the safety of the method. The errors are doubtless largest in the mercury, while the oxygen carries the sum of the errors of the other five elements.

For the formula $(C_{10}H_{14}N_2O_2)_2(HI)_2(HgI_2)_2.H_2O$, the centesimals stand as follows:

	Calculated.	Found, mean.
C	19.91	20.85
H	2.21	2.35
N	2.32	2.41
Hg	24.88	23.25
I	47.34	47.47
O	3.31	3.68

Mr. Kebler observed the solubilities of the iodomercurates of pyridine and quinoline. These bases are both precipitated from their salts by Mayer's reagent, the precipitation being permanent when the reagent is in excess. Both precipitates are slightly soluble in water. Iodomercurate of pyridine is soluble in alcohol, dilute or absolute, and in hot acetic acid of 40 per cent. It melts at 102°–103° C. Iodomercurate of quinoline is still more soluble in acetic acid.

A sample of pyridine was fractionated until a portion was secured with a constant boiling-point of 114.5° C. for several successive distillations. The hydrochloride of this body, acidulated with hydrochloric acid, was treated at 25° C. with the decinormal solution of potassium mercuric iodide, short of excess, the precipitate washed on a filter at 60° C., and dried at 60° C. to a constant weight. Elementary analysis by the procedure already describes gave figures, in triplicate, as follows, compared with the formula, $C_5H_5N(HgI_2)_2$:

	Calculated.	Found. I.	II.	III.	Mean.
C	6.08	5.85	5.86	5.91	5.87
N	1.42	2.10	1.83	1.62	1.85
I	51.44	50.00	52.16	51.12	51.09
Hg	40.57	41.26	39.86	40.49	40.53
H	0.49	0.79	0.29	0.89	0.66

The hydrogen figures were obtained by difference.

A sample of synthetically-prepared quinoline was fractionated until a portion was obtained giving a constant boiling-point under constant low pressure, the last distillation being done in an atmos-

phere of dry hydrogen. The purified quinoline was treated for precipitation of iodomercurate, exactly as done for the pyridine compound. The precipitation was at 25° C., and the reagent not in excess. The centesimals were as follows, compared with the formula $C_9H_7NHgI_2$:

	Calculated.	Found. I.	II.	Mean.
C	18.47	18.72	18.59	18.65
N	2.39	2.54	2.91	2.72
I	43.35	43.51	43.12	43.31
Hg	34.20	34.00	33.79	33.89
H	1.54	1.23	1.59	1.43

The reactions of pyridine with mercuric chloride, mercuric iodide, and metatungstic acid, upon pyridine, were obtained by Koenigs and Geigy in 1884.[1]

All discussion of the contrast between the pyridine and the quinoline iodomercurate is reserved for better data and further study. The question is of importance to inquiries of the linking of mercury, iodine and nitrogen, in alkaloids in general.

UNIVERSITY OF MICHIGAN, *August*, 1892.

TRIMETHYLXANTHINE AND SOME OF ITS DERIVATIVES.[2]

BY MOSES GOMBERG.

The constitutional formula of caffeine, as first proposed by Medicus[3] and afterwards modified by E. Fischer,[4] explains quite clearly all the decompositions of the alkaloid. Its probable correctness becomes so much more apparent when we remember that the formulæ for theobromine, theophyline[5] and xanthine have been derived from that of caffeine, and all the experimental evidence goes to corroborate the formulæ of the lower homologues of caffeine.

[1] Ber. d. chem. Ges. **17**, 594.

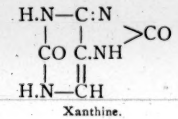
[2] Read by A. B. Prescott at the Rochester Meeting of the American Association for the Advancement of Science, August, 1892.

[3] Ann. Chem. (Liebig) **175**, 250.

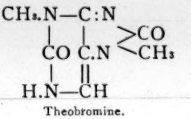
[4] *Ibid.* **215**, 253.

[5] Zischr. physiol. Chem. **13**, 298.

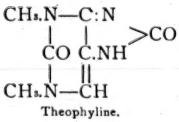
The formulæ for the four compounds are:



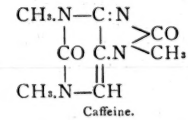
Xanthine.



Theobromine.



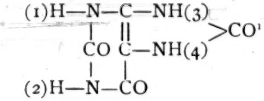
Theophyline.



Caffeine.

The presence of three imide groups in xanthine explains how it and its homologues, theobromine and theophyline, can act as acids, and how caffeine has no acid properties whatever.

But it is on comparing the properties and reactions of xanthine and its homologues on one hand, with those of uric acid and some of its homologues on the other, that the correct conception expressed by the respective formulæ becomes so much more clear. Like that of xanthine, the formula of uric acid,



explains how it and its homologues, mono-, di-, and even trimethyl-uric acid, can still act as acids, forming at least with some bases salt-like bodies. It is for this reason that we have a tetramethyl-uric acid,[2] while no corresponding derivative of xanthine has been prepared.

The important difference between these two classes of compounds is still more strikingly brought out by the difference in their behavior towards hydrochloric acid under pressure:

$$\begin{cases} C_5H_4N_4O_2 + 6H_2O = NH_3 + NH_3 + NH_3 + NH_2.CH_2.CO_2H + H.CO_2H + 2CO_2^3 \\ C_6H_4N_4O_3 + 5H_2O = NH_3 + NH_3 + NH_3 + NH_2.CH_2.CO_2H + 3CO_2^4 \end{cases}$$

[1] Medicus: Ann. Chem. (Liebig) **175**, 230; E. Fischer: Ber. d. chem. Ges. **18**, 1776.
[2] E. Fischer: Ber. d. chem. Ges. **18**, 1784.
[3] E. Schmidt: Ann. Chem. (Liebig) **217**, 309.
[4] Strecker: *Ibid.* **146**, 142.

$$\begin{cases} C_8H_3(CH_3)_3N_4O_2 + 6H_2O = NH_2.CH_3 + NH_3 + NH_3 + \\ \quad NH.CH_2.CH_2.CO_2H + H.CO_2H + 2CO_2{}^1 \\ C_8H_3(CH_3)_3N_4O_2 + 5H_2O = NH_2.CH_3 + NH_3 + NH_3 + \\ \quad NH.CH_2.CH_2.CO_2H + 3CO_2{}^2 \end{cases}$$

$$\begin{cases} C_8H(CH_3)_3N_4O_2 + 6H_2O = NH_2.CH_3 + NH_2.CH_3 + NH_3 + \\ \quad NH.CH.CH_2.CO_2H + H.CO_2H + 2CO_2{}^3 \\ C_8H(CH_3)_3N_4O_2 + H_2O = \text{a crystalline compound, undeter-} \\ \quad \text{mined.}{}^4 \end{cases}$$

$$\begin{cases} \text{---------- ----------} \\ C_8(CH_3)_4N_4O_2 + 5H_2O = NH_2.CH_3 + NH_2.CH_3 + NH_2.CH_3 + \\ \quad NH.CH_2.CH_2.CO_2H + 3CO_2\ (?){}^5 \end{cases}$$

It appeared to the writer of some scientific interest to make an attempt to prepare a tetramethylxanthine. Besides the physiological interest attached to it as a probable new alkaloid, the object was to determine, if possible, whether this new compound would break up under the influence of hydrochloric acid and pressure, with liberation of three substituted-ammonia molecules to one of free ammonia, as might be expected from the present structural formula of xanthine. Although the results are far from being complete, yet the writer begs leave to report them such as they are, however little light they throw upon the subject in question. The work done so far can be best treated of under separate sections:

I. Iodocaffeine.

II. Action of sodium upon a mixture of an alkyl-halogen and a halogen-caffeine.

III. Action of zinc ethyl upon halogen-caffeine.

IV. Action of potassium cyanide upon chlorocaffeine.

I. There are known two halogen-caffeines, $C_8H_9ClN_4O_2$ and $C_8H_9BrN_4O_2$. As both are very stable compounds, it was thought best to prepare a derivative more sensitive to the action of reagents; this, it was expected, would be found in iodocaffeine.

The ordinary method of introducing iodine into organic compounds,—namely, by treating the corresponding chloro- or bromo-

[1] For theobromine, *vid.* E. Schmidt: *loc. cit.*, p. 298.

[2] For β-dimethyluric acid [(3) and (4)], *vid.* E. Fischer: Ber. d. chem. Ges. **18**, 1780.

[3] E. Schmidt: *loc. cit.*, p. 278.

[4] E. Fischer: *loc. cit.*, p. 1783.

[5] *Ibid.*, p. 1784: "Decomposes like the other homologues, with liberation of methyl-amine, *but no ammonia.*"

compound with an iodide of a light metal,—proved in this case unsuccessful. First chlorocaffeine, then the bromo-compound, were heated with 2 to 3 times the theoretical quantity of sodium iodide, potassium iodide, and calcium iodide in alcoholic solutions, in flasks with inverted condensers. When filtered the liquids gave no indication of the presence of either chlorine or bromine.

The same trials were repeated by substituting sealed tubes for open flasks, at a temperature of 140°–150° C., but even then no change could be detected. An attempt was made to raise the temperature; at 195°–200° C., about two-thirds of the alcohol employed was converted into ether, by the hydriodic acid from the dissociation of the metallic iodide; all the halogen-caffeine was generally found to have been reduced to caffeine.

The experiments were repeated by using exactly theoretical quantities in absolute alcohol, but with similar results. Absolute ether was then substituted for the alcohol. The tube was heated for two weeks at 150° C., but no change could be noticed, except that some iodine was set free.

An attempt was next made to prepare iodocaffeine by direct substitution of iodine for hydrogen, in a manner analogous to that employed by E. Fischer in preparing chlorocaffeine. But the dark residue, obtained by heating a mixture of iodine and caffeine in chloroform, was at once decomposed on addition of water, with formation of caffeine. It was decided to isolate this addition-compound and examine it more closely; after several trials the following method was found to give satisfactory results:

Dry caffeine is dissolved in a suitable amount of anhydrous chloroform. The flask is connected with a Soxhlet's extraction-apparatus, in which a good supply of iodine is placed—about four times the quantity of caffeine employed. After several hours' action the chloroform is distilled, the dark residue is heated in an oil-bath to 140°–150° C., whereby the largest part of the excess of iodine is sublimed. The product is then powdered and placed in the extraction-apparatus. It is first exhausted for several hours with chloroform to remove the unchanged caffeine, then with carbon bisulphide till the latter shows only a very slight iodine coloration. The product is a dark granular powder, with no odor of iodine, almost insoluble in carbon bisulphide, ether, chloroform; quite permanent on exposure to air, but on addition of water or alcohol it immediately decomposes with liberation of caffeine and iodine.

An elementary analysis gave the following results:

I. 0.9614 gram substance gave 0.4237 gram CO_2 and 0.1318 gram H_2O.

II. 0.3127 gram substance gave 0.1367 gram CO_2 and 0.0404 gram H_2O.

III. 0.4283 gram substance gave 0.1772 gram CO_2 and 0.0603 gram H_2O.

IV. 0.2106 gram substance gave 13.2 cc. N at 22° C. and 741.7 mm.

V. 0.3762 gram substance gave 23.2 cc. N at 22° C. and 741.7 mm.

VI. 0.4303 gram substance gave 27.3 cc. N at 25.3° C. and 729.9 mm.

VII. 0.5461 gram substance gave 0.4110 gram I.

VIII. 0.4607 gram substance gave 0.3482 gram I.

IX. 0.1628 gram substance gave 0.1238 gram I.

Combustions I and II were made by mixing the substance with copper oxide; combustion III, by using lead chromate.

Determinations VII and VIII were made by suspending the substance in water, treating with sulphur dioxide, and precipitating with silver nitrate. Determination IX was made by igniting the substance with lime.

	Calculated for $C_8H_{10}N_4O_2.I_2$.	Found.						Mean.
C	11.61	(I)	12.02	(II)	11.63	(III)	11.29	11.65
H	1.21		1.52		1.43		1.56	1.50
N	6.77	(IV)	6.93	(V)	6.82	(VI)	7.10	6.95
I	76.54	(VII)	75.26	(VIII)	75.57	(IX)	76.03	75.62

When suspended in water and treated with standard sodium thiosulphate the compound gave, as a mean of four estimations, 60.05 per cent. of iodine, showing that only four atoms of iodine are addition-atoms, the fifth having an entirely different function in the molecule.

In 1865 Tilden[1] obtained an "iodocaffeine" by dissolving caffeine in a weak alcoholic solution of hydriodic acid. The compound, according to Tilden, is very unstable, does not stand the heat of a water-bath, and is to be dried *in vacuo;* its formula is $(C_8H_{10}N_4O_2.HI).I_2 + H_2O$. But the compound prepared by the writer was dried at 95° C., cannot possibly have any water of cystallisation, and can hardly contain hydriodic acid as such.

[1] J. Chem. Soc. (new series) **8**, 99.

Heated for three hours in an air-bath at 70° C., 1.3218 grams of the substance lost only 0.0026 gram=0.19 per cent.; then on being again heated for three hours at 100°–110° C. it began to fuse slightly and lost 0.0129 gram=0.97 per cent. Awaiting further experimental proof, the writer is inclined to think that the compound might be a tetraiodide of iodocaffeine, the latter being very unstable.

II. The action of sodium upon a mixture of an alkyl-halogen and a halogen-caffeine did not give satisfactory results. A great drawback to the successful application of this reaction was found in the fact that the halogen-caffeines are hardly at all soluble in ether, benzene, toluene, rhigolene, etc.

III. The action of compounds of zinc with aliphatic radicles upon halogen compounds is generally accompanied with great evolution of heat, and a diluent, such as ether, is usually employed. But neither chloro- nor bromocaffeine was acted upon when treated in this way. After distilling the ether the temperature had to be raised above 100° C. to distil the last portion of zinc ethyl. The solid residue was washed with hot water, but the latter showed only a minute trace of chlorine or bromine.

Pure zinc ethyl was next employed. This reagent was freshly prepared by Gladstone and Tribe's[1] method, and distilled directly from the couple upon a quantity of chlorocaffeine, the receiver being immersed in ice-water. The mixture was then gradually heated to 160° C. and kept at this temperature for 3 hours. At about 120° the halogen-compound all dissolved in the zinc ethyl, but on cooling separated again as a heavy white crust. The excess of zinc ethyl was distilled directly upon some bromocaffeine. The residue was exposed to air for some time, then washed with hot water; but the latter gave only a slight test for chlorine. The residue was proved to be unchanged chlorocaffeine, which is almost insoluble in water.

The bromocaffeine was now subjected to the same treatment as described above. It also dissolved at about 120° C., but to a dark solution, and on cooling separated as a dark-yellow crust. After removing the excess of zinc ethyl by distillation, the residue was washed with hot water, and the latter was found to contain a large amount of bromine.[2] The attempt to obtain the compound pure

[1] J. Chem. Soc. 1879, **1**, 571.

[2] Bromocaffeine, like the chloro-compound, is almost insoluble in water, and does not precipitate silver nitrate.

enough for analysis failed. The experiment was repeated on a larger scale, but the compound has not yet been obtained of sufficient purity.

IV. By the action of potassium cyanide upon a halogen-caffeine it was expected to prepare cyanocaffeine; this by further special treatment should give methyl-caffeine. 15 grams potassium cyanide were dissolved in about 600 cc. of 80 per cent. alcohol, the hot solution was filtered, and to it were added 30 grams of chlorocaffeine, which easily dissolved on heating. The hot solution was once more filtered and then boiled with an inverted condenser for three hours. By that time a heavy, yellowish sediment came down. This was separated by filtration, washed with hot alcohol, dilute hydrochloric acid, and finally hot water; dried at 110° C. The yield was 21 grams. Subjected to elementary analysis it gave the following results:

	Calculated for $C_8H_9(CN)N_4O_2$.	Found. I.	II.	III.
C	49.31	46.27	...	...
H	4.12	5.13	...	...
N	31.96	...	29.70	30.10

The experiment repeated with 25 grams chlorocaffeine and 12 grams potassium cyanide yielded 18 grams, with similar results upon analysis.

Fearing that the material prepared as described might contain some impurities, several portions of it were dissolved separately in large quantities of glacial acetic acid, in which solvent the compound is but slightly soluble—and solution filtered while hot. On cooling, white, somewhat crystalline deposits separated. They were washed with warm glacial acetic acid, then with hot water, and finally dried at 125° C. The different precipitates were analysed independently, and furnished the following results:

I. 0.2735 gram gave 0.4587 gram CO_2 + 0.1239 gram H_2O.
II. 0.3878 gram gave 0.6563 gram CO_2 + 0.1764 gram H_2O.
III. 0.2695 gram gave 0.4492 gram CO_2 + 0.1284 gram H_2O.
IV. 0.1453 gram gave 38.3 cc. N at 17° C. and 736 mm.
V. 0.2013 gram gave 53 cc. N at 20° C. and 736 mm.
VI. 0.2208 gram gave 60.5 cc. N at 21.5° C. and 736.5 mm.
VII. 0.2260 gram gave 61 cc. N at 26° C. and 739 mm.
VIII. 0.1574 gram gave 40.9 cc. N at 18° C. and 745 mm.
IX. 0.0856 gram gave 22.3 cc. N at 22° C. and 744.2 mm.

Combustions IV, V and VI were made in a platinum boat. In combustion VII a copper boat was used and the substance was mixed with copper oxide in the boat. In VIII and IX a closed tube was employed and the substance was mixed with the copper oxide in the tube.

Calculated in percentage the results are:

	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
C	45.73	45.92	45.50	...	...	...	...	...	...
H	5.03	5.05	5.28	...	...	...	...	...	...
N	...	...	...	29.60	29.18	30.11	30.09	29.41	28.89

It is clear from an inspection of the results that the compound is not *cyanocaffeine*. According to E. Fischer,[1] prolonged boiling of bromocaffeine with a dilute alcoholic solution of potassium cyanide results in the production of a small quantity of *amidocaffeine*. It is reasonable to suppose that chlorocaffeine would act towards potassium cyanide as the bromo-compound does. But the writer is inclined to think that Fisher's compound is not *amidocaffeine*, for chlorocaffeine at least does not give that, as is seen on comparing the results with the theoretical figures:

	Calculated for $C_8H_9(NH_2)N_4O_2$.	Found (mean of the foregoing 9 determinations).
C	45.90	45.70
H	5.30	5.12
N	33.50	29.56

It would also be difficult to find an explanation for the formation of an amido-compound in this case. Potassium cyanate, nearly always present, could account for the production of only a small amount of amidocaffeine, while the yield obtained was in both cases about 85 per cent. of the theoretical value. Of course, if we assume that *isocyanocaffeine* is the first product of the reaction, then we can easily see how it can give rise to amidocaffeine. But there is no reason to make such an assumption.

On the other hand, however, if we suppose the action to be normal we can find an explanation. We may assume that the product is a half-saponified cyanocaffeine, resulting probably thus:

$$C_8H_9(Cl)N_4O_2 + KCN = C_8H_9(CN)N_4O_2 + KCl$$
$$C_8H_9(CN)N_4O_2 + H_2O = C_8H_9(CO.NH_2)N_4O_2.$$

[1] Ann. Chem. (Liebig) **215**, 266.

Further saponification would give:

$$C_8H_9(CO.NH_2)N_4O_2 + H_2O = C_8H_9(CO.OH)N_4O_2 + NH_3.$$

The comparison of the theoretical figures with the results obtained would especially suggest that reaction.

	Calculated for $C_8H_9(CO.NH_2)N_4O_2$.	Found.
C	45.56	45.70
H	4.56	5.12
N	29.54	29.56

The production of an acid-amide from a cyano-compound without the help of either acid or alkali is somewhat rare but not altogether unusual; so when benzyl chloride, $C_6H_5.CH_2Cl$, is heated with potassium cyanide, besides the normal product benzyl cyanide, $C_6H_5.CH_2CN$, phenylacetamide, $C_6H_5CH_2.CO.NH_2$, is also produced.

The supposition of this being the case here might be justified (1) by the results of the elementary analysis of the compound; (2) by the probability of the reaction; and (3) by the behavior of the compound. Although all attempts to prepare carboxy-caffeine have so far failed, yet some of the results are suggestive. When the compound described is treated with potash in alcohol, or with strong hydrochloric acid, in sealed tubes at 130° C., or with H_2SO_4(1 : 1) at 100° C., it breaks up into *caffeine and carbon dioxide*, pointing to the intermediate formation of carboxy-caffeine, which, however, under the conditions employed is not stable.

In conclusion I must express my thanks to Mr. A. W. Augir, who kindly helped me in determining the elementary composition of the compounds used and prepared.

University of Michigan, *August*, 1892.

NOTES OF WORK FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF VIRGINIA.

No. XVIII.

Communicated by F. P. DUNNINGTON.

151.—ANALYSIS OF CUPROPLUMBITE FROM BUTTE CITY, MONTANA.

BY J. T. DE BELL.

This mineral was received with some other ores from the Canton Copper Co., of Baltimore. It is amorphous, of bluish-gray black color, lustre sub-metallic, fracture uneven, fuses with spurting, sp. grav.=5.39, hardness=2.5, and upon analysis presents the following composition:

		Atomic Ratio.
Copper	61.32	10.51
Lead	18.97	1.00
Sulphur	17.77	6.01
Silica (quartz)	1.58	...
	99.64	

Which gives approximately the formula, $5Cu_2S.PbS$.

In Dana's Mineralogy[1] mention is made of three varieties of cuproplumbite, viz:

$$Cu_2S.2PbS$$
$$2Cu_2S.PbS$$
$$3Cu_2S.PbS$$

The specimen before us adds, therefore, another member to this series.

The question is also raised[2] as to whether these compound sulphides are to be classified with galena or with chalcosite.

If we consider galena as having sp. grav.=7.5, and imagine in each 6 molecules that 5 atoms of lead (5Pb) are replaced by 10 atoms of copper ($5Cu_2$) without altering the aggregate bulk of the molecules, this will give the sp. grav.=5.45.

If, on the other hand, we take chalcosite (sp. grav.=5.7) and similarly in 6 molecules replace Cu_2 by Pb, then its calculated sp. grav.=6.16.

[1] 6th edition, page 51.

[2] Loc. cit.

Now the mineral under discussion has the sp. grav.=5.39, and when this is corrected for the quartz present it gives 5.43, which nearly coincides with the above calculated sp. grav. 5.45 obtained when it is regarded as built in the form of galena. From this it appears that the mineral is to be classed with isometric galena.

152.—ANALYSIS OF ELECTRIC CALAMINE FROM WYTHE COUNTY, VIRGINIA.

By Archibald Jones.

This mineral is found on New River, in the mines belonging to and now worked by the Bertha Zinc Company, of Pulaski, Va.

Some years ago, an analysis made in this laboratory was published,[1] showing that the snow-white opaque sheets of ore from this mine consist of electric calamine containing a slight excess of zinc oxide and water.

Encrusting the material above-mentioned there is occasionally found a layer of bright, transparent, colorless crystals 1 to 2 mm. in thickness. Some of this layer was carefully removed and afforded the following results: specific gravity=3.40; hardness=4.9; and composition:

		Calculated for $Zn_2SiO_4 + H_2O$.
Silica	25.33	25.0
Zinc oxide	67.15	67.5
Water	7.47	7.5
	99.95	100.0

From which it appears that these crystals present a peculiarly pure specimen of electric calamine.

153.—ANALYSES OF TSCHERNOZEM OR BLACK SOIL.

By F. P. Dunnington and T. C. Whitlock.

With several specimens of typical soils from British America, kindly furnished me by Dr. Geo. M. Dawson, F. R. S., Ottawa, Can., there was one from the prairie land of Red River, taken at Rossers, about 15 miles west of Winnipeg, Manitoba, which presented so marked similarity in appearance to the black soil of

[1] Chemical News, 731.

Russia that I thought it would prove interesting to make such comparison of the general nature of these soils as is expressed by a chemical analysis of the two.

Among some specimens of Tschernozem or Black Earth of Russia sent to me by Prof. Nicolas Menschutkin, of St. Petersburg, darkest in color, was one from the land of M. Naroushkin, District Balashoff, Government Saratoff. These two specimens, which are so similar in appearance that they are not to be distinguished by the eye, have been analysed by Mr. Whitlock with the following results:

	Manitoba.	Russia.
Sand,	59.82	53.71
Silica, amorphous,	5.45	12.80
Ferric oxide,	4.00	4.13
Alumina,	7.14	6.04
Titanic oxide,	.64	.63
Lime,	.61	.75
Magnesia,	.61	.21
Sulphuric oxide,	.03	.06
Carbonic oxide,	.37	.02
Phosphoric oxide,	.13	.16
Potash (with trace of soda)	1.91	1.97
Organic matter,	12.49	14.91
Containing humus,[1]	(.45)	(.44)
" total nitrogen,	(.44)	(.31)
Water,	6.86	5.04
	99.76	100.43

This analysis of the Russian black earth fairly agrees with published analyses of other specimens of it.

With the exception of the amounts of carbonic acid and of the proportion of the silica which is amorphous, the composition of these two specimens is almost identical.

The peculiarly large amounts of organic matter and nitrogen, as well as of the principal constituents of the ash of plants, lime, potash and phosphoric acid, are all to be noted and accord with the well-known exceeding fertility of each of these soils.

The soil from Manitoba is described by Dr. Dawson[2] as "spread with great uniformity over the Red River valley, a wide prairie on the first or lowest prairie level of the Northwestern country. It has a depth of say one to four feet, and consists of the superfi-

[1] Soluble in ammonia.

[2] In a private letter and in report on the Geology of 49th Parallel, 1875.

cially modified parts of the sediments of a later glacial or post-glacial lake, which at greater depths are found in the form of well-bedded silts. . . . Within the province of Manitoba it covers an area of not less than several thousand square miles. . . . The surface is a dark mould, composed of the same material as the subsoil, but mingled with much vegetable matter. . . . The uniform fertility of this soil cannot be exaggerated."

The Tschernozem or Black Earth of Russia has long been famous by reason of the heavy crops which it has, in many localities, annually produced for almost a century. Prof. Krassnof, in a paper[1] upon this subject, describes it as distributed over the steppes of the southeastern portion of European Russia. He concludes that this black color is due to an accumulation of vegetable matter from the herbaceous plants of the poorly drained steppes of the post-glacial deposits which overlie the loessoid clays, so difficultly permeable to water. At the close of this paper he draws attention to the close correspondence which exists between the climate of Russsia and that of the prairie land of Minnesota (much of which lies in the Red River valley) and suggests the probable similarity of the soils of these regions.

The above analyses make it to appear that the Tschernozem and the soil of the Red River prairies are similar in chemical composition; they occur in the same latitude, with the same general relief and climate, and from the above quoted authorities are judged to have a similar geological history; may they not therefore be properly considered as of the one variety of soil, "Black Earth"?

154.—ANALYSIS OF WASHING POWDERS.

By W. J. Kinney, W. H. Wenger and F. P. D.

The various washing powders used as substitutes for soap present a conspicuous example of substances in general use which are usually employed with little understanding of their nature or composition.

The four samples selected are of such powders for washing clothes, etc., largely sold in Virginia, and a knowledge of their composition will prove of interest to many. Analysis afforded the following results:

[1] Proc. Geolog. Soc. America, Washington Meeting, 1891, page 68.

	A.	B.	C.	D.
Sodium carbonate	45.2	26.9	49.2	46.6
Fatty acids	26.4	44.0	25.6	25.7
Combined soda	3.1	3.4	3.5	2.6
Fine sand	...	16.3	...	...
Water	23.7	8.8	19.1	24.9
	98.4	99.4	97.4	99.8

A portion of this water is necessarily in the soap, and the remainder is with the sodium carbonate, which has in each case been partially dried. The soaps used do not contain rosin. Not a trace of borax was present.

We may therefore describe these substances as generally composed of a mixture of soap and dried washing soda, both powdered.

While a small amount of such powders may properly be employed, in conjunction with soap, to remove the "hardness" of the water when washing textile fabrics, yet the substitution of any such powders for soap must result in a gradual corrosion of cotton, linen or woolen goods.

Borax might be employed in place of soda in the above preparations with great advantage (to all except the manufacturer), since it has no corrosive action upon such fabrics, and, while it removes all hardness from the water, is also an excellent detergent.

155.—SOLUBILITY OF CREAM OF TARTAR IN DILUTED ALCOHOL.

By W. H. Wenger.

The solubility of cream of tartar in water, wine and other liquids at varying temperatures has been carefully studied by M. Chancel; but no figures are given for other dilute alcohol than that of 10.5 per cent.

With a view to use in some analytical processes the following determinations were made.

Measured amounts of a saturated aqueous solution of cream of tartar were mixed with the calculated amount of 92 per cent. alcohol, to give residual liquids of the desired strength of alcohol. These were repeatedly shaken and allowed to stand for some days at a temperature of approximately 25° C., and then measured amounts of the liquids were filtered off and titrated with deci-

normal solution of sodium hydrate, using phenolphthalein as the indicator. The following results were obtained:

Grams of cream of tartar dissolved in 1000 cc. of—

Alcohol of 90 per cent.	.15
80 "	.19
70 "	.30
60 "	.41
50 "	.79
40 "	1.32
30 "	1.96
20 "	3.01
10 "	4.51
Water	5.75

It is proposed to enlarge this table by obtaining these estimates of solubility for other temperatures.

156.—ANALYSES OF KALE AND TURNIP SALAD AND OKRA.

By J. D. Tinsley.

Both of the specimens of salad were gathered and analysed in April. The kale was weighed promptly after cutting. The turnip salad was cut some hours before being weighed, when it had wilted somewhat, hence the larger proportion of solid matter which it contains in the "fresh" leaves (this was not noted until the analysis was nearly completed). The specimen of okra pods was cut in August, weighed immediately, sliced, dried and analysed the next April.

The analyses were conducted by the scheme presented by the American Association of Agricultural Chemists and afforded the following:

100 parts of the "fresh" plant.	Kale salad.	Turnip salad.	Okra pods.
Water by air-drying	88.34	77.78	83.07
Water at 212°	1.32	2.27	1.64
Solid matter	10.34	19.95	15.29
Solid matter containing:			
Pure ash	1.22	2.62	.91
Ether extract	.65	1.59	.29
Crude fibre	1.15	1.57	1.44
Crude starch, gum, etc.	3.43	8.07	10.12
Crude protein	3.89	6.10	2.53
Nitrogen total,	.62	1.12	.41
Nitrogen albumenoid,	.25	.68	.28

Composition of, Dried at 100°.	Kale salad.	Turnip salad.	Okra pods.
Ash, pure	11.83	13.15	6.65
Ether extract	6.35	8.01	2.02
Crude fibre	11.16	7.93	9.39
Crude protein	37.75	35.12	18.37
Starch, etc., by diff.	32.91	35.79	63.57

No doubt the dietetic value of these vegetables is not to be reckoned solely by the nutrients which they contain, yet in view of their very general use as food, their composition is a matter of interest. The large amount of protein in the salads, as shown in the latter table, is of special note, in that it is even greater than in dried beans or peas, which are ordinarily instanced as most highly nitrogenous of the vegetable foods of man.

157.—ANALYSIS OF STATUARY MARBLE FROM RUTLAND, VERMONT.

By Jno. T. De Bell.

This marble is now extensively quarried for building purposes, and being quite uniform as well as moderately fine in texture, it is also employed for statuary other than the finest work. It is occasionally marred by pale greenish seams, showing minute foreign crystals.

A sample free of visible seams was furnished by Edw'd V. Valentine, Esq., of Richmond, Va., and this upon analysis showed the following impurities in 100 parts:

SiO_2	0.167
Fe_2O_3 and Al_2O_3	0.062
Na_2O	0.065
K_2O	0.004
MgO	0.079

corresponding to the composition:

Calcium carbonate	99.536
Magnesium carbonate	0.174
Other impurities	0.290

An examination of Carrara marble shows it to contain:

Na_2O(K_2O trace) . . . . . 0.046 per cent.

hence lime made from the latter presents but little superiority to that from Vermont marble, when employed in analysis for the separation of magnesia from alkalies.

158.—ANALYSES OF GLAUCONITE FROM HANOVER COUNTY, VIRGINIA.

By M. B. Corse and Chas. Baskerville.

The extensive beds of greensand marl which occur in portions of Hanover, Chesterfield, Caroline, Prince William, Spottsylvania and Stafford counties vary greatly in depth and in the amount of potash contained. Among the richest of these deposits are beds from 3 to 6 feet in depth upon the farm of Maj. Winston, near Hanover Court House, which have a total potash content of from 1 to 2 per cent.

By washing the marl the clean glauconite was separated, and possessed the following properties and composition. Under the microscope it appears as rounded green grains interspersed with seams of quartz and with some spots of a much deeper green color than the main portion. It is almost insoluble in strong acids, but after gentle ignition, by which it loses water and becomes brown, it may be completely gelatinised by hydrochloric acid. Ignited in a closed glass tube, it becomes black. Of A, sp. grav. = 2.83; fusibility = 4.5.

Samples A and B were similarly obtained from different beds in the same vicinity; analysis of these affords:

	A.	B.
SiO_2 combined	47.45	43.34
Quartz	2.76	8.22
Al_2O_3	7.33	6.62
Fe_2O_3	12.03	15.16
FeO	9.43	8.33
MgO	2.90	.95
CaO	.57	.62
K_2O	5.75	4.15
Na_2O	.42	1.84
H_2O	9.85	10.32
	98.49	99.55

This considerable variation in the composition of the glauconite from contiguous beds but confirms the opinion that the mineral is a mixture. Any mechanical separation of the dark and pale green portions of these specimens would be impossible.

159.—ANALYSIS OF PARANTHITE FROM CLAY COUNTY, NORTH CAROLINA.

By W. N. Berkeley.

This mineral was found by Mr. C. G. Memminger when superintendent of the mines of the Hiawassee Corundum Company, situated five miles southeast of Hayesville, N. C., who describes its occurrence as follows:

About ten pounds of the mineral, in rounded masses, of a few ounces weight each, was found at No. 2 mine, in a pocket near the centre of a six-foot vein, consisting of decomposed albite, corundophilite, chlorite, vermiculite and other chloritic minerals, lying between chrysolite and hornblende gneiss. Corundum is scattered through the vein-matter in grains and crystals varying in size from minute particles up to pieces weighing one-half a pound. During a year's work only this one pocket of the mineral was observed.

It consists of rounded crystalline lumps of a bluish-grey color. Sp. grav. = 2.75; hardness = 5.5; and upon analysis afforded the following:

		Oxygen Ratio.
SiO_2	47.54	4.7
Al_2O_3	34.03	3.
CaO	17.23	1.
Na_2O	1.82	
H_2O	1.02	

Since no chlorine is present, this mineral scarcely admits of being considered as a mixture of meionite and marialite, as it has been suggested by Tschermak[1] to express the composition of all such minerals; but it is rather to be designated as paranthite, in that its analysis closely resembles that of the mineral from Pargas,[2] to which Wolff assigned this name.

[1] Dana's Mineralogy, 6th ed., p. 466.
[2] Dana's Mineralogy, 5th ed., p. 319.

PROTEIDS OF THE FLAX-SEED.

By Thomas B. Osborne.

The extensive use, as a cattle-food, of flax-seed partly freed from oil by expression or extraction, under the name of linseed-meal, renders desirable as complete a knowledge as possible of its chemical composition. As the chief value of this feed is due to the large amount of albuminoids contained in it, a careful study of these bodies is important from a practical as well as from a scientific standpoint.

Although extended examination of the literature of the vegetable proteids has been made, the writer has nowhere found any investigation relating to the proteids contained in this seed. A study of these proteids was accordingly undertaken, the results of which are given in this paper.

I.—Preliminary Experiments.

Ground flax-seed freed from oil by extraction with benzine or ether yields to both water and solutions of sodium chloride a large proportion of its proteid matter. After complete exhaustion with water, 10-per cent. sodium chloride brine extracts a further portion of proteid. Neither water nor salt solution removes all the proteids, for there always remains in the extracted residue more or less proteid soluble in dilute potash-water, as well as some nitrogenous substance, probably proteid, which the latter reagent fails to remove. Of the substance soluble in water or sodium chloride solution, a very large part is a globulin which has been separated in a crystalline form and prepared in a state of purity.

1. *Reactions of the Aqueous Extract.*

Very dilute acetic acid added to the aqueous extract of the meal gave a precipitate soluble in a slight excess of acid, but reprecipitated upon adding sodium chloride. Boiling produced a coagulum and great turbidity. Filtered from the coagulum, the hot solution gave a heavy precipitate with acetic acid, soluble in an excess, but reprecipitated upon adding sodium chloride. Saturation with ammonium sulphate completely precipitated the proteids. Satur-

ation with sodium chloride gave no precipitate. Dilution with water was without effect. Prolonged dialysis produced but a trifling separation of the proteid. On standing for some days a considerable deposit, soluble in sodium chloride solution, separated. Heated slowly in a double water-bath, the extract became turbid at 50° C., a few flocks separating at 60°. Filtered after heating to 65° and again heated, the solution became turbid a second time at 70°, and flocks in minute amount formed at 78°. Filtered at 80°, a third turbidity developed at 82°, some flocks forming at 88°. Filtered at 90° and boiled, a coagulum was produced.

2. *Reactions of the Sodium Chloride Extract.*

An extract of the meal made with 10-per cent. sodium chloride solution gave the following results:

Dilute acetic acid produced a precipitate not noticeably soluble in an excess of dilute acid, but completely soluble in an excess of strong acid when hot and nearly so when cold. Boiling gave a very little coagulum, the filtrate from which, with dilute acetic acid, yielded a heavy precipitate. Saturation with ammonium sulphate completely precipitated the proteids. Saturation with sodium chloride caused a slight precipitate. Dilution with water gave a small precipitate after standing which, in some cases, was crystalline. Heated slowly, the clear solution became turbid at 45°, flocks appearing at 58°. Filtered at 63°, the solution became turbid again at 65°, and a few flocks developed at 76°. Heated to 80° and filtered, a turbidity appeared at 85° and flocks at 88°. Filtered at 90°, a coagulum formed on boiling, the filtrate from which gave a precipitate with dilute acetic acid which was soluble in a large excess of strong acid.

3. *Reactions of the Sodium Chloride Solution of the Separated Globulin.*

A solution prepared by dissolving the globulin in 10-per cent. sodium chloride brine had the following properties, which are here stated so that the reactions of the extract may be better understood.

Dilute acetic acid gave a heavy precipitate mostly soluble in a large excess of strong acid in the cold, and completely soluble on heating. Boiling yielded a little coagulum, the filtrate from which

gave a heavy precipitate on adding acid. Saturation with ammonium sulphate caused a complete precipitation of the proteid. Saturation with sodium chloride produced a small precipitate. Dilution with water caused a small precipitate. Heated slowly the solution became turbid at 57°, flocks forming at 67°. Filtered at 72°, it became turbid again at 75° and developed flocks at 80°. Filtered at 85°, turbidity began at 85°, flocks forming at 88°. Filtered and boiled, a coagulum formed, the filtrate from which gave a large precipitate with acetic acid, soluble in a considerable excess of hot acid.

The reactions of these three solutions differed in the following respects:

With very dilute acetic acid the aqueous extract gave a precipitate soluble in a slight excess of acid, whereas the other solutions gave a precipitate soluble only in a large excess of strong acid. The cause of this difference lies in the fact that vegetable globulins are soluble in a very slight excess of acid in the absence of salts, but are insoluble in their presence, the insolubility increasing with the amount of salts.

Saturation with sodium chloride gave no precipitate in the aqueous extract, while in the other solutions it produced a small precipitate. This difference might be explained by the supposition that the salt solution extracted globulins insoluble in the salt solutions derived from the mineral constituents of the seed, were it not for certain observations on the coagulation-points soon to be described.

The heat-coagulation temperatures of these solutions are given in the following table:

HEAT-COAGULATION-POINTS OF THE

	Aqueous extract.	10-per cent. sodium-chloride extract.	10-per cent. sodium-chloride solution of the globulin.
1st. Turbidity,	50°	45°	57°
Flocks,	60°*	58°*	67°*
Filtered at	63°	63°	72°
2d. Turbidity,	70°	65°	75°
Flocks,	78°†	76°†	80°†
Filtered at	80°	85°	85°
3d. Turbidity,	82°	85°	85°
Flocks,	88°	88°	88°
Filtered at	90°	90°	90°
Boiling gave a	coagulum.	coagulum.	coagulum.

* Greatest in amount. † Least in amount.

The two extracts agree well with each other, but the first and second coagulation-points of the solution of the globulin are 10° higher. This difference is probably due to the fact that the extract had a decided acid reaction, while the solution of the globulin was perfectly neutral. These heat-coagulation-points leave little doubt that they belong to the same bodies in all three solutions.

These coagulation-points indicate that besides the globulin separated from the extract in the crystalline form there are other globulins present in small amounts, which coagulate below boiling. This is shown by the fact that when a strong solution of the crystalline globulin in 10-per cent. brine is saturated with sodium chloride and, after filtering and diluting so that the solution again contains 10-per cent. sodium chloride, it is tested by carefully heating, the solution remains perfectly clear until heated to 73°, when it becomes turbid and separates a minute amount of flocculent matter at 82°. Heated to boiling, after filtering off this very minute coagulum, only an insignificant amount of the substance coagulates, but on adding acid a very heavy precipitate is thrown down. Furthermore, when the slight precipitate formed by saturating the 10-per cent. sodium chloride solution of the globulin with salt is washed with a saturated brine (so as to remove the dissolved globulin) and then taken up in a little water (so that the resulting solution of sodium chloride may contain 10 per cent. of this salt), the coagulable globulins are thus separated from the crystalline globulin. Coagulation-tests of a solution thus prepared gave the following results, which are given together with those obtained with a 10-per cent. sodium chloride solution of the original mixed globulins:

COAGULATION-POINTS OF THE SALT SOLUTION OF THE

	Precipitate produced by saturation with sodium chloride.	Original mixed globulin.
Turbidity,	59°*	57°*
Flocks,	68°	67°
Filtered at	72°	72°
Turbidity,	78°†	75°†
Flocks,	83°	80°
Filtered at	86°	85°
Turbidity,	..	85°
Flocks,	89°	88°
	Increased very little on boiling. Some precipitate with acid.	Increased on heating to boiling, a considerable coagulum being formed. A very large precipitate with acid.

* Most in amount.

† Least in amount.

It is thus seen that the globulins coagulating below 90° are almost entirely removed by saturation with sodium chloride and that their amount is extremely small; accordingly, no further attempt was made to separate them, there being no probability of obtaining preparations fit for analysis.

A coagulation-test of the filtered and salt-saturated solution of the globulin showed a turbidity of 73° and minute flocks at 82°, indicating that the globulin coagulating at this temperature is only partly removed from the solution by saturation with sodium chloride.

These coagulable globulins were found to be much more soluble than the crystallisable globulin, in dilute solutions of sodium chloride; for on extracting the separated globulins with ½-per cent. sodium chloride brine, nearly the whole of these coagulable proteids was removed.

II.—Extraction of the Globulin.

1. *Extraction with pure water at* 20° *C.*—The ground flax-seed was freed from oil by extracting with benzine, and from the greater part of the outer coating of the seed by sifting through a fine sieve. The meal thus prepared, when air-dry, contained 8.40 per cent. of nitrogen—equivalent to 52.50 per cent. of proteids, assuming these to contain 16 per cent. of nitrogen. 100 grams of this meal were extracted with distilled water. The filtered extract had a strong yellow color, distinct acid reaction, and was very slightly turbid. The residue was then twice again extracted with distilled water, and the three extracts, after filtering, were united and saturated with pure ammonium sulphate. These operations were completed within 24 hours. The very abundant precipitate thus produced was collected on a filter and, after the solution had all run off, was removed from the paper and dissolved in about 800 cc. of water. This solution was then filtered perfectly clear and placed in a dialyser. After 24 hours a considerable precipitate had formed, which was seen under the microscope to consist entirely of perfectly formed octahedral crystals. After dialysing five days this solution was free from chlorides, the precipitate was then filtered off and the clear filtrate returned to the dialyser. Under the microscope the precipitate was found to be almost entirely composed of crystals, but a few spheroids were found among them. The deposit adhering to the paper contained some

very large particles which appeared crystalline to the naked eye. The precipitate was washed with water; dilute alcohol gradually increased in strength to absolute alcohol; then with ether, and dried over sulphuric acid. This preparation (**1**) which still retained its crystalline character, weighed 10.5 grams. It was therefore 10.5 per cent. of the meal and had the following composition:

FLAX-SEED GLOBULIN.—*Preparation* **1.**

		Ash-free.
Carbon	51.29	51.42
Hydrogen	7.04	7.06
Nitrogen	18.57	18.61
Sulphur	0.76	0.76
Oxygen	..	22.15
Ash	0.25	..
		100.00

Ash.—0.6412 gram substance, dried at 110° C., gave 0.0016 gram ash = 0.25 per cent.

Carbon and hydrogen.—0.3264 gram dried substance gave 0.6138 gram CO_2 = 51.29 per cent. C, and 0.2067 gram H_2O = 7.04 per cent. H.

Nitrogen.—0.3504 gram dried substance gave 53.95 cc. N at 15° C. (barometer 773.0 mm. at 23° C.) = 18.57 per cent.

Sulphur.—0.8655 gram dried substance gave 0.0478 gram $BaSO_4$ = 0.0066 gram S = 0.76 per cent.

The filtrate from **1** was further dialysed for five days and, by proceeding in the manner just described, 0.4 gram of preparation **2** was obtained in the form of spheroids which, without correction for ash, yielded 18.74 per cent. of nitrogen.

FLAX-SEED GLOBULIN.—*Preparation* **2.**

Nitrogen 18.74

Nitrogen.—0.2620 gram substance, dried at 110° C., gave 41.22 cc. N at 13° C. (barometer 758.5 mm. at 25° C.) = 18.74 per cent.

2. *Extraction with pure water at* 40° *C.*—40 grams of flax-seed meal containing 8.56 per cent. of nitrogen were treated with 200 cc. of distilled water at 40° for 5 hours. The solution was then made up to a liter and allowed to stand over-night at the temperature of the room. The extract was filtered off and, after washing the residue with water, was saturated with ammonium sulphate. The precipitate formed was filtered and the filtrate

very carefully tested for proteids with the biuret test, but none were found. The ammonium sulphate precipitate was dissolved in water and a little sodium chloride solution and, after removing a small insoluble residue by filtration, the clear solution was placed in a dialyser. After five days this solution was found to be free from chlorides and contained a deposit consisting of crystals and spheroids. After washing the latter with alcohol and ether and drying over sulphuric acid, it weighed 4 grams, being 10 per cent. of the flax-seed meal employed.

FLAX-SEED GLOBULIN.—*Preparation* **3.**

		Ash-free.
Carbon	51.47	51.69
Hydrogen	..	..
Nitrogen	18.62	18.70
Sulphur	0.73	0.73
Oxygen	..	..
Ash	0.43	..

Ash.—0.5134 gram substance, dried at 110° C., gave 0.0022 gram ash = 0.43 per cent.

Carbon and hydrogen.—0.3535 gram dried substance gave 0.6672 gram CO_2 = 51.47 per cent. C; hydrogen was lost.

Nitrogen.—0.3107 gram dried substance gave 47.49 cc. N at 11.5° C. (barometer 771.5 mm. at 22° C.) = 18.62 per cent.

Sulphur.—0.7610 gram dried substance gave 0.0404 gram $BaSO_4$ = 0.056 gram S = 0.73 per cent.

3. *Extraction with sodium chloride solution after exhausting with water at* 20° *C.*—The residue of the flax-seed meal after extracting with cold water, as described, was next treated twice with 10-per cent. sodium chloride solution, the extract filtered clear and saturated with ammonium sulphate. The precipitate so produced was filtered off, removed from the paper, dissolved in 10-per cent. sodium chloride brine, filtered from a small insoluble residue and the clear solution dialysed. After 24 hours dialysis crystals and spheroids appeared, and after five days the solution was filtered from the precipitate, which was then washed with water, alcohol—at first dilute and afterwards absolute—and finally with ether, and dried over sulphuric acid. It weighed 2 grams and agreed quite well in composition with preparation **1**, as may be seen from the following analysis:

FLAX-SEED GLOBULIN.—*Preparation* 4.

		Ash-free.
Carbon	50.92	51.17
Hydrogen	6.87	6.90
Nitrogen	18.86	18.95
Sulphur	0.88	0.88
Oxygen	..	22.10
Ash	0.49	..
		100.00

Ash.—0.5492 gram substance, dried at 110° C., gave 0.0027 gram ash = 0.49 per cent.

Carbon and hydrogen.—0.3105 gram dried substance gave 0.5797 gram CO_2 = 50.92 per cent. C, and 0.1921 gram H_2O = 6.87 per cent. H.

Nitrogen.—0.3791 gram dried substance gave 59.1 cc. N at 13° C. (barometer 771.0 mm. at 27° C.) = 18.86 per cent.

Sulphur.—0.4030 gram dried substance gave 0.0261 gram $BaSO_4$ = 0.0036 gram S = 0.88 per cent.

4. *Extraction with sodium chloride solution after extracting with water at* 40° *C.*—The residue of flax-seed meal, after extracting with water at 40°, as described on page 12, was twice treated with 10-per cent. sodium chloride brine and the clear, filtered extracts dialysed free from chlorides. A perfectly crystallised preparation resulted, which, when treated in the usual manner, weighed 2.7 grams, equal to 6.8 per cent. of the flax-seed meal. Dried at 110° and analysed, the following results were obtained:

FLAX-SEED GLOBULIN.—*Preparation* 5.

		Ash-free.
Carbon	50.79	51.46
Hydrogen	6.85	6.94
Nitrogen	18.17	18.41
Sulphur	0.89	0.90
Oxygen	..	22.29
Ash	1.30	..
		100.00

Ash.—0.6355 gram substance, dried at 110° C., gave 0.0083 gram ash = 1.30 per cent.

Carbon and hydrogen.—0.3865 gram dried substance gave 0.7197 gram CO_2 = 50.79 per cent. C, and 0.2382 gram H_2O = 6.85 per cent. H.

Nitrogen.—0.3824 gram dried substance gave 57.15 cc. N at 11.5° C. (barometer 770 mm. at 22° C.) = 18.17 per cent.

Sulphur.—0.6527 gram dried substance gave 0.0425 gram $BaSO_4$ = 0.0058 gram S = 0.89 per cent.

5. *Direct extraction with sodium chloride solution.*—100 grams of the flax-seed meal were extracted three successive times with 20-per cent. sodium chloride solution, and the clear, filtered, bright-yellow extracts were united and saturated with ammonium sulphate. The precipitate produced was filtered off, removed from the paper and dissolved in 10 per cent. sodium chloride brine, and this solution, after filtering from an insoluble residue, was submitted to dialysis. After five days the solution, free from chlorides, was filtered from the separated globulins and returned to the dialyser. The precipitate was treated in the same manner as in the preceding. It amounted to 11.65 per cent. of the flax-seed meal when dried over sulphuric acid, still retained its crystalline form and had the following composition:

FLAX SEED GLOBULIN.—*Preparation* 6.

		Ash-free.
Carbon	51.22	51.32
Hydrogen	6.85	6.87
Nitrogen	18.57	18.60
Sulphur	0.82	0.82
Oxygen	..	22.39
Ash	0.20	..
		100.00

Ash.—0.8020 gram substance, dried at 110° C., gave 0.0016 gram ash = 0.20 per cent.

Carbon and hydrogen.—0.4535 gram dried substance gave 0.8517 gram CO_2 = 51.22 per cent. C, and 0.2796 gram H_2O = 6.85 per cent. H.

Nitrogen.—0.3786 gram dried substance gave 58.33 cc. N at 15° C. (barometer 772.7 mm. at 23° C.) = 18.57 per cent.

Sulphur.—1.0265 grams dried substance gave 0.0612 gram $BaSO_4$ = 0.0084 gram S = 0.82 per cent.

The filtrate from this precipitate yielded only an insignificant amount of substance on further dialysis.

6. *Direct extraction with a saturated sodium chloride solution.*—As saturation of the sodium chloride extract of flax-seed meal with salt precipitated nearly all of the coagulable proteids, a preparation of the globulin was made by extracting 40 grams of the meal with a saturated brine and submitting the clear filtered extract to dialysis until all the salt was removed and the globulin was deposited in the dialyser in the form of spheroids. This deposit was filtered off, washed with water, alcohol and ether, and found to weigh 3.7 grams, being 9.25 per cent. of the meal. Dried at 110°, it had the following composition:

FLAX-SEED GLOBULIN.—*Preparation* 7.

		Ash-free.
Carbon	51.12	51.59
Hydrogen	7.21	7.27
Nitrogen	18.17	18.34
Sulphur	..	22.80
Oxygen	..	
Ash	0.92	..
		100.00

Ash.—0.5977 gram substance, dried at 110° C., gave 0.0055 gram ash = 0.92 per cent.

Carbon and hydrogen.—0.3985 gram dried substance gave 0.7478 gram CO_2 = 51.12 per cent. C, and 0.2587 gram H_2O = 7.21 per cent. H.

Nitrogen.—0.2407 gram dried substance gave 35.83 cc. N at 7° C. (barometer 760.6 mm. at 23° C.) = 18.17 per cent.

7. *The globulin extracted by direct treatment of the flax-seed meal with dilute potash solution.* (*a*) *Deportment of the globulin toward* $\frac{2}{10}$ *per cent. potash solution.*—It was necessary before extracting the meal with potash solution to determine, if possible, whether this reagent would alter the properties and composition of the globulin. Five grams of preparation 6 were dissolved in 25 cc. of $\frac{2}{10}$ per cent. caustic potash solution and the perfectly clear but somewhat brownish-colored liquid was diluted with 375 cc. of distilled water. A nearly colorless solution resulted, which was very carefully neutralised with extremely dilute hydrochloric acid. This threw down a snow-white precipitate soluble in the most minute excess of either acid or alkali, as well as in solutions of sodium chloride. A dilute warm solution of sodium chloride saturated with this precipitate and filtered from the excess of substance deposited the globulin, on cooling, in well-formed octahedral crystals. The remainder of the precipitate was washed with water, alcohol and ether, and, after drying over sulphuric acid, weighed 4.05 grams and had the following composition:

FLAX-SEED GLOBULIN.—*Preparation* 8.

		Ash-free.	Average of Preps. 1–10.
Carbon	51.52	51.67	51.48
Hydrogen	6.90	6.92	6.94
Nitrogen	18.74	18.79	18.60
Sulphur	0.82	0.82	0.81
Oxygen	..	21.80	22.17
Ash	0.26	..	..
		100.00	100.00

Ash.—0.7344 gram substance, dried at 110° C., gave 0.0019 gram ash = 0.26 per cent.

Carbon and hydrogen.—0.3403 gram dried substance gave 0.6428 gram CO_2 = 51.52 per cent. C, and 0.2115 gram H_2O = 6.90 per cent. H.

Nitrogen.—0.3294 gram dried substance gave 50.94 cc. N at 11.5° C. (barometer 766.7 mm. at 20° C.) = 18.74 per cent.

Sulphur.—0.6945 gram dried substance gave 0.0415 gram $BaSO_4$ = 0.0015 gram S = 0.82 per cent.

It is thus seen that this globulin retained its properties unchanged, without conversion into "alkali-albuminate," by solution in weak caustic potash. Its composition is in close agreement with the average of the preparations already described, the slight difference being most probably due to the greater purity of the recrystallised globulin. Grübler also obtained crystalline preparations from the globulin of the squash-seed after solution in weak potash-water.

Ritthausen has been severely criticised by physiological chemists because he used dilute caustic alkalies in extracting proteids, although he proved in many cases that the products thus prepared retained their solubility in sodium chloride solution and had the same composition as the globulins extracted directly by sodium chloride brine and precipitated therefrom by dilution.

(*b*) *Direct extraction of the flax-seed meal with dilute potash solution.*—11 grams of flax-seed meal were next treated directly with 55 cc. $\frac{2}{10}$-per cent. caustic potash solution and the mixture made up to 1100 cc. with distilled water. This solution had a distinct alkaline reaction toward litmus. After filtration 1000 cc. of the clear liquid, equal to an extract from 10 grams of substance, were saturated with ammonium sulphate, and the precipitate produced filtered off and dissolved as far as possible in sodium chloride brine. The resulting solution was filtered clear and dialysed until chlorides were removed. The substance separated by dialysis, under the microscope was seen to consist of spheroids and imperfectly developed crystals. The deposit filtered off, washed with water, alcohol and ether and dried over sulphuric acid weighed 1.75 grams, being 17.5 per cent. of the air-dry flax-seed meal, or when both meal and globulin were dried at 110°, equal to 16.5 per cent. This substance had the following composition:

FLAX-SEED GLOBULIN.—*Preparation* 9.

Carbon	51.23	51.53
Hydrogen	6.90	6.94
Nitrogen	18.35	18.45
Sulphur	0.73	0.73
Oxygen	..	22.35
Ash	0.60	..
	0.60	100.00

Ash.—0.4839 gram substance, dried at 110° C., gave 0.0029 gram ash = 0.60 per cent.

Carbon and hydrogen.—0.3259 gram dried substance gave 0.6122 gram CO_2 = 51.23 per cent. C, and 0.2023 gram H_2O = 6.90 per cent. H.

Nitrogen.—0.3533 gram dried substance gave 53.15 cc. N at 11.5° C. (barometer 772. mm. at 21° C.) = 18.35 per cent.

Sulphur.—0.2751 gram dried substance gave 0.0152 gram $BaSO_4$ = 0.0021 gram S = 0.73 per cent.

Another preparation was made in nearly the same manner, as follows: 25 grams of flax-seed meal containing 8.64 per cent. of nitrogen were treated with 100 cc. of $\frac{2}{10}$-per cent. caustic potash solution and 700 cc. of distilled water added. The resulting mixture not being alkaline, 100 cc. more $\frac{2}{10}$-per cent. potash solution were added, imparting to the extract a distinct alkaline reaction. The solution was then filtered from the insoluble residue and the latter washed well with water. The filtrate and washings were united and saturated with ammonium sulphate. The precipitate obtained was then thoroughly extracted with 10-per cent. sodium chloride solution, and after filtering placed in a dialyser, where it remained two weeks. The deposit which formed consisted of spheroids. Filtered off and washed with alcohol and ether and dried over sulphuric acid, the product weighed 1.56 grams, being 6.2 per cent. of the meal taken. This substance was composed as follows:

FLAX-SEED GLOBULIN.—*Preparation* 10.

		Ash-free.
Carbon	51.28	51.50
Hydrogen	6.92	6.95
Nitrogen	18.29	18.37
Sulphur, Oxygen	..	23.18
Ash	0.44	..
		100.00

Ash.—0.6730 gram substance, dried at 110° C., gave 0.0030 gram ash = 0.44 per cent.

Carbon and hydrogen.—0.2761 gram dried substance gave 0.5192 gram CO_2 = 51.28 per cent. C, and 0.1722 gram H_2O = 6.92 per cent. H.

Nitrogen.—0.3003 gram dried substance gave 46.27 cc. N at 10.5° C. (barometer 749. mm. at 22° C.) = 18.29 per cent.

The preceding analyses are here brought together for comparison.

Extracted by water at 20°.

	1	2	3
Carbon	51.42	..	51.69
Hydrogen	7.06	..	..
Nitrogen	18.61	18.74	18.70
Sulphur	0.76	..	0.73
Oxygen	22.15	..	.
	100.00		

Extracted by salt solution.

	After water at 20°. 4	After water at 40°. 5	Direct. 6	Direct. 7
Carbon	51.17	51.46	51.32	51.59
Hydrogen	6.90	6.94	6.87	7.27
Nitrogen	18.95	18.41	18.60	18.34
Sulphur	0.88	0.99	0.82	} 22.80
Oxygen	22.10	22.29	22.39	
	100 00	100.00	100.00	100.00

Extracted by $\frac{2}{10}$-per cent. potash solution.

	Prep. 6 dissolved in potash and precipitated. 8	9	10	Average. 1-10
Carbon	51.67	51.53	51.50	51.48
Hydrogen	6.92	6.94	6.95	6.94
Nitrogen	18.79	18.45	18.37	18.60
Sulphur	0.82	0.73	} 23.18	0.81
Oxygen	21.80	22.35		22.17
	100.00	100.00	100.00	100.00

These results agree so closely with the analyses of the globulin obtained from squash-seed that there can be no doubt that the two proteids are identical. This agreement is shown by the following figures:

	Flax.	Squash-seed.		
	Osborne.	Chittenden and Hartwell.[1]	Ritthausen.[2]	Grübler.[3]
Carbon	51.48	51.60	51.61	51.48
Hydrogen	6.94	6.97	7.00	6.76
Nitrogen	18.60	18.80	..	18.14
Sulphur	0.81	1.01	..	0.96
Oxygen	22.17	21.62	..	22.66
	100.00	100.00		100.00

III.—The "Albuminate" derived from the Globulin.

In several cases where the ammonium-sulphate precipitate was treated with sodium chloride solution so long as anything was removed, there remained a considerable amount of undissolved proteid—evidently an "albuminate" derived from the globulin. When this substance was dissolved in dilute sodium carbonate solution and precipitated on neutralisation, a precipitate was obtained, which, on filtering off and washing with water, alcohol and ether, was analysed after drying at 110°. The composition of the various products thus obtained was very varied; in some cases being the same as that of the globulin, and in others quite different, especially in respect to nitrogen. It is then evident that, by the methods employed in making some of these preparations for analysis, great changes in the proteid were caused.

1. *The "Albuminate" dissolved in Sodium Carbonate Solution and precipitated on Neutralisation with Acid.*

When the ammonium-sulphate precipitate of the 10-per cent. sodium chloride extract from which preparation **4** was taken, was treated with brine, a portion remained undissolved. This was washed with 10-per cent. sodium chloride brine, dissolved in ½-per cent. sodium carbonate solution, precipitated by careful neutralisation with hydrochloric acid, the precipitate filtered off, washed with water, alcohol and ether, and dried over sulphuric acid. This substance weighed 0.6 gram and was the insoluble, or "albuminate," form of the globulin. It contained the following amount of nitrogen:

Flax-seed Proteid.—*Preparation* **11.**

		Ash-free.
Nitrogen	18.63	18.84
Ash	1.14	..

[1] J. Physiol. **11**, 440. [2] J. prakt. Chem. **25**, 136. [3] J. prakt. Chem. **44**, 369.

Ash.—0.3145 gram substance, dried at 110° C., gave 0.0036 gram ash = 1.14 per cent.

Nitrogen.—0.2478 gram dried substance gave 38.22 cc. N at 13° C. (barometer 769.5 mm. at 26° C.) = 18.63 per cent.

When the ammonium-sulphate precipitate from which preparation **6** was obtained was extracted with 10-per cent. sodium chloride solution, a considerable amount of proteid remained undissolved. This was then dissolved in ½-per cent. sodium carbonate solution and, after filtering clear, was precipitated on neutralisation with dilute hydrochloric acid. This precipitate, after washing with water, alcohol and ether, weighed, air dry, 4.4 grams and was marked Preparation **12**. It had the following composition:

FLAX-SEED PROTEID.—*Preparation* **12**.

			Ash-free.		
	I.	II.	I.	II.	Average.
Carbon	51.29	51.14	51.56	51.41	51.49
Hydrogen	7.16	6.98	7.19	7.01	7.10
Nitrogen	17.21	16.95	17.30	17.04	17.17
Sulphur	1.03	..	1.04	..	1.04
Oxygen	..	..	22.91	..	23.20
Ash	0.53	..	..	..	..
			100.00		100.00

Ash.—0.6587 gram substance, dried at 110° C., gave 0.0035 gram ash = 0.53 per cent.

Carbon and hydrogen, I.—0.3580 gram dried substance gave 0.6733 gram CO_2 = 51.29 per cent. C, and 0.2317 gram H_2O = 7.16 per cent. H.

II.—0.3391 gram dried substance gave 0.6357 gram CO_2 = 51.14 per cent. C, and 0.2128 gram H_2O = 6.98 per cent. H.

Nitrogen, I (Dumas' method).—0.3900 gram dried substance gave 55.36 cc. N at 13° C. (barometer 772.0 mm. at 26° C.) = 17.21 per cent.

II (Kjeldahl method).—0.8312 gram dried substance gave ammonia = 17.15 cc. HCl solution (1 cc. = 0.00822 gram N) = 16.95 per cent. N.

Sulphur.—0.5507 gram dried substance gave 0.0428 gram $BaSO_4$ = 0.0059 gram S = 1.03 per cent.

In another extraction the ammonium-sulphate precipitate, when thoroughly washed with 10-per cent. brine, left a considerable insoluble residue. This was dissolved in 1-per cent. sodium carbonate solution and, after filtering clear, was divided into two parts. One part was precipitated on adding very dilute hydrochloric acid to slightly acid reaction; the precipitate was filtered off and washed with water. After washing out the salts the pre-

cipitate began to dissolve. It was then washed with alcohol and ether. On drying at 110° it was found to have the following composition:

FLAX-SEED PROTEID.—*Preparation* **13.**

			Ash-free.		
	I.	II.	I.	II.	Average.
Carbon	50.69	50.66	51.91	51.88	51.90
Hydrogen	6.58	6.59	6.74	6.73	6.74
Nitrogen	11.26	11.36	11.83	11.73	11.78
Sulphur } Oxygen }	..	..	29.72	29.86	29.58
Ash	2.35	..	..	..	..
			100.00	100.00	100.00

Ash.—0.3994 gram substance, dried at 110° C., gave 0.0094 gram ash = 2.35 per cent.

Carbon and hydrogen, I.—0.3570 gram dried substance gave 0.6635 gram CO_2 = 50.69 per cent. C, and 0.2110 gram H_2O = 6.58 per cent. H.

II.—0.3994 gram dried substance gave 0.7418 gram CO_2 = 50.66 per cent. C, and 0.2375 gram H_2O = 6.59 per cent. H.

Nitrogen, I.—0.3187 gram dried substance gave 31.22 cc. N at 17° C. (barometer 755.6 mm. at 20° C.) = 11.46 per cent.

II.—0.3193 gram dried substance gave 32.42 cc. N at 17° C. (barometer 755.6 mm. at 20° C.) = 11.56 per cent.

The other portion of the solution was saturated with ammonium sulphate, and carbon dioxide passed through it. But little precipitate being thus produced which could be removed by filtration, a very slight excess of hydrochloric acid was added, which caused a precipitate to fall. This was filtered off, washed thoroughly with water, alcohol and ether. On washing with water a little of the substance dissolved. Dried at 110°, the following results were obtained on analysis:

FLAX-SEED PROTEID.—*Preparation* **14.**

			Ash-free.		
	I.	II.	I.	II.	Average.
Carbon	50.61	50.43	50.97	50.80	50.89
Hydrogen	6.87	6.71	6.92	6.76	6.84
Nitrogen	16.25	16.28	16.36	16.39	16.38
Oxygen } Sulphur }	..	..	25.75	26.05	25.89
Ash	0.71	..	..	..	..
			100.00	100.00	100.00

Ash.—0.4100 gram substance, dried at 110° C., gave 0.0029 gram ash = 0.71 per cent.

Carbon and hydrogen, I.—0.3482 gram dried substance gave 0.6462 gram CO_2 = 50.61 per cent. C, and 0.2154 gram H_2O = 6.87 per cent. H.

II.—0.3728 gram dried substance gave 0.6893 gram CO_2 = 50.43 per cent. C, and 0.2250 gram H_2O = 6.71 per cent. H.

Nitrogen, I.—0.3271 gram dried substance gave 46.1 cc. N at 17° C. (barometer 758.0 mm. at 21° C.) = 16.25 per cent.

II.—0.3479 gram dried substance gave 49.13 cc. N at 17° C. (barometer 758.0 mm. at 21 C.) = 16.28 per cent.

On a second time extracting with 10-per cent. sodium chloride solution the flax-seed meal from which the two preceding preparations had been obtained, and saturating this second extract with ammonium sulphate, it was found that a considerable part of the precipitate produced by the ammonium sulphate had become insoluble in 10-per cent. salt solution. After thoroughly washing with brine, the insoluble residue was dissolved in 1-per cent. sodium carbonate solution and the liquid filtered perfectly clear. This was then neutralised with the greatest care. When the precipitate separated from the solution and was apparently completely thrown down, it was found that the solution turned blue litmus paper quite red, but on drying the paper on carefully cleaned platinum it became blue again. On the other hand red litmus paper was unaffected by the solution until dried on platinum, when it became blue. This reaction was not due to the precipitate, for on filtering the solution perfectly clear the same result was obtained.

This filtrate was then carefully tested for further precipitable substance by adding extremely dilute hydrochloric acid, and also dilute sodium carbonate solution, but neither gave any more precipitate. Sodium chloride was also added to the filtrate in order to precipitate any acid-globulin present, but none was found. After washing the precipitate with water it was found to dissolve. The whole was therefore dissolved in distilled water and a solution obtained which behaved exactly like a solution of the crystallised globulin in dilute acid, giving a precipitate, on adding a little sodium chloride, which did not dissolve in a larger amount of salt. The solution was dialysed until all chlorides were removed. It then separated in a gelatinous mass adhering to the parchment. This was scraped off, rinsed into a beaker and allowed to settle over-night. In the morning it had gathered in a mass at

the bottom of the vessel. It was filtered off, washed with absolute alcohol which did not dehydrate it completely, then with ether, and dried over sulphuric acid, when it became horny and translucent. Dried at 110° it had the following composition:

FLAX-SEED PROTEID.—*Preparation* **15.**

			Ash-free.		Average.
	I.	II.	I.	II.	
Carbon	51.75	51.50	51.81	51.56	51.69
Hydrogen	6.88	6.86	6.88	6.86	6.87
Nitrogen	17.42	..	17.44	..	17.44
Sulphur	0.81	..	0.81	..	0.81
Oxygen	..	..	23.06	..	23.19
Ash	0.12	..	..	..	..
			100.00		100.00

Ash.—0.8320 gram substance, dried at 110° C., gave 0.0010 gram ash = 0.12 per cent.

Carbon and hydrogen, I.—0.3823 gram dried substance gave 0.7255 gram CO_2 = 51.75 per cent. C, and 0.2365 gram H_2O = 6.88 per cent. H.

II.—0.3559 gram dried substance gave 0.6720 gram CO_2 = 51.50 per cent. C, and 0.2195 gram H_2O = 6.86 per cent. H.

Nitrogen.—0.4134 gram dried substance gave 62.66 cc. N at 17° C. (barometer 755.6 mm. at 20° C.) = 17.42 per cent.

Sulphur.—0.7860 gram dried substance gave 0.0464 gram $BaSO_4$ = 0.0064 gram S = 0.81 per cent.

Again, 200 grams of flax-seed meal were extracted with 10-per cent. sodium chloride solution, the clear extract saturated with ammonium sulphate and the precipitate thus obtained thoroughly exhausted with brine. The undissolved residue was then treated with ½-per cent. sodium carbonate solution, the solution filtered, neutralised with dilute hydrochloric acid, and the resulting precipitate filtered off, washed with alcohol and ether, and dried over sulphuric acid. It weighed 5.9 grams, and when dried at 110° gave the following results on analysis:

FLAX-SEED PROTEID.—*Preparation* **16.**

		Ash-free.
Carbon	51.59	51.72
Hydrogen	6.80	6.82
Nitrogen	18.35	18.40
Sulphur	0.96	0.96
Oxygen	..	22.10
Ash	0.26	..
		100.00

Ash.—0.6091 gram substance, dried at 110° C., gave 0.0016 gram ash = 0.26 per cent.

Carbon and hydrogen.—0.4015 gram dried substance gave 0.7596 gram CO_2 = 51.59 per cent. C, and 0.2458 gram H_2O = 6.80 per cent. H.

Nitrogen.—0.3432 gram dried substance gave 51.76 cc. N at 11.5° C. (barometer 771.8 mm. at 24° C.) = 18.35 per cent.

Sulphur.—0.7000 gram dried substance gave 0.0495 gram $BaSO_4$ = 0.0067 gram S = 0.96 per cent.

200 grams of the flax-seed meal were treated with water, and the fine meal in suspension decanted from the gummy husks. Sodium chloride was then added to the aqueous extract, and the solution filtered clear. This solution was then saturated with ammonium sulphate, and the large precipitate filtered off, pressed between filter-papers in order to remove as much as possible of the adhering solution, and dissolved in 10-per cent. sodium chloride brine. A very considerable part of the substance was now found to be insoluble in sodium chloride solution. This was therefore filtered off, washed with brine to free it from the soluble globulin, and then with water. The substance so obtained was divided into two parts. One was dissolved in ½-per cent. sodium carbonate solution, the resulting liquid filtered clear and neutralised with $\frac{2}{10}$-per cent. hydrochloric acid. Before complete neutralisation the substance began to precipitate. After neutralising as exactly as possible, the precipitate was filtered off and washed with distilled water. After the salts present were removed the precipitate began to dissolve in water, showing the properties of an acid-globulin. The substance was then washed with alcohol and ether, and, dried over sulphuric acid, weighed 6.66 grams. Dried at 110°, the following results were obtained on analysis:

FLAX-SEED PROTEID.—*Preparation* **17.**

		Ash-free.
Carbon	51.61	52.04
Hydrogen	6.80	6.85
Nitrogen	17.12	17.26
Sulphur	..	..
Oxygen	..	..
Ash	0.84	..

Ash.—0.5500 gram substance, dried at 110° C., gave 0.0046 gram ash = 0.84 per cent.

Carbon and hydrogen.—0.4277 gram dried substance gave 0.8094 gram CO_2 = 51.61 per cent. C, and 0.2618 gram H_2O = 6.80 per cent. H.

Nitrogen.—0.3854 gram dried substance gave 52.95 cc. N at 6° (barometer 773.6 mm. at 21° C.) = 17.12 per cent.

2. *Solution of the "Albuminate" in Warm Sodium Chloride Solution.*

The other portion of the insoluble proteid last described was treated with 10-per cent. sodium chloride solution heated to 50°, and was found to dissolve almost completely. On cooling, a considerable amount of substance separated, and was seen under the microscope to consist of octahedral crystals. This preparation was washed with water, alcohol and ether, and weighed, air-dry, 1.4 grams. Dried at 110° it had the following composition:

FLAX-SEED GLOBULIN.—*Preparation* **18.**

		Ash-free.
Carbon	50.98	51.14
Hydrogen	6.79	6.81
Nitrogen	18.24	18.30
Sulphur } Oxygen }	..	23.75
Ash	0.32	..
		100.00

Ash.—0.3687 gram substance, dried at 110° C., gave 0.0012 gram ash = 0.32 per cent.

Carbon and hydrogen.—0.4261 gram dried substance gave 0.7965 gram CO_2 = 50.98 per cent. C, and 0.2604 gram H_2O = 6.79 per cent. H.

Nitrogen.—0.3215 gram dried substance gave 46.87 cc. N at 5° C. (barometer 773.5 mm. at 19° C.) = 18.24 per cent.

The filtrate from preparation **18** was then dialysed till free from salt, and the crystalline deposit resulting was filtered off, washed with water, alcohol and ether. It weighed, air-dry, 2.1 grams, and had the following composition when dried at 110°:

FLAX-SEED GLOBULIN.—*Preparation* **19.**

		Ash-free.
Carbon	51.30	51.39
Hydrogen	6.90	6.91
Nitrogen	18.54	18.57
Sulphur } Oxygen }	..	25.13
Ash	0.19	..
		100.00

Ash.—0.3759 gram substance, dried at 110° C., gave 0.0007 gram ash = 0.19 per cent.

Carbon and hydrogen.—0.3858 gram dried substance gave 0.7258 gram CO_2 = 51.30 per cent. C, and 0.2395 gram H_2O = 6.90 per cent. H.

Nitrogen.—0.2866 gram dried substance gave 42.63 cc. N at 6° C. (barometer 773.6 mm. at 21° C.) = 18.54 per cent.

The table here given will facilitate a comparison of the preceding analyses:

	11	12	13	14	15	16	17	18	19
Carbon	..	51.49	51.90	50.89	51.69	51.72	52.04	51.14	51.39
Hydrogen	..	7.10	6.74	6.84	6.87	6.84	6.85	6.81	6.91
Nitrogen	18.84	17.17	11.78	16.38	17.44	18.40	17.26	18.30	18.57
Sulphur	..	1.04	29.58	25.89	0.81	..	..	23.75	..
Oxygen	..	23.20			23.19	..	..		
		100.00	100.00	100.00	100.00			100.00	

It is clear that many of the above analyses represent no definite substance and that, in most cases, by the treatment employed extensive changes, both in the properties and composition of the proteid, have been brought about. Preparations **13**, **14**, **15** and **17** had the properties of an acid-globulin, being insoluble in water containing a small amount of salts, and soluble in pure distilled water containing a trace of acid, and also in alcohol; whereas preparations **11**, **12** and **16** were undissolved by distilled water. Preparations **18** and **19** were in all respects like the original globulin, and show therefore that the so-called "*albuminate*" of Weyl is something very different from an *alkali-albuminate*.

These analyses also show that the insoluble form of the globulin is the same in composition as the globulin itself. What the real cause of the change in solubility may be is not clear, but it is not improbable that the globulins form compounds with water which are soluble in solutions of salts, and that when separated from solution these hydrates break down and an insoluble form results. Or it may be that the globulins form loose compounds with other substances in the solution, as, for instance, coloring matters or salts, and that on warming, these are broken up and the original globulin is restored. A like conversion of an insoluble into a soluble globulin was accomplished by the writer with the so-called albuminate similarly obtained from the sodium-chloride extract of the oat-kernel.[1]

[1] This Journal **14**, 212.

IV.—The Proteids remaining in Solution after removing the Globulin.

After separating the globulin by dialysis and filtration, the clear liquid can be heated to boiling without producing a coagulum, but if concentrated to a small volume a coagulation gradually takes place. If the dialysed liquid be treated with about 2 per cent. of sodium chloride, and a little hydrochloric acid be added, a precipitate is produced consisting of a proteid resembling an albumin in its solubility in water, but like a globulin in that, when precipitated by salt and acid, it forms an acid compound soluble in water nearly or wholly free from salts. On the other hand it is thrown out of such solutions upon neutralisation with sodium carbonate.

Similar substances were found in the extracts of the maize kernel and, in a paper on the proteids of that seed, were described as albumins.[1] Together with this body there is precipitated more or less proteose, resembling closely in composition the deutero-vittelose obtained by Chittenden and Hartwell from the crystallised proteid of the squash-seed.[2] After separating the coagulable proteids by concentrating their solution to a small volume and by filtration, there was always found in the filtrate more or less proteid matter of the character of the proteoses and peptones.

1. *Proteids coagulating on boiling.*

Two extractions were made, each with 100 grams of flax-seed meal; one (portion A) was treated with water, and the other (B) with 20-per cent. sodium chloride solution. Both these extracts were precipitated by saturating with ammonium sulphate, dissolving the precipitates in water containing sodium chloride, and dialysing until no more globulin was precipitated. Especial care was taken to make the separation of globulin as complete as possible, by the use of river-water remarkably free from dissolved salts. The clear solutions, free from globulin, were then concentrated by boiling and filtered from coagulum.

These preparations were washed with water, alcohol and ether, and dried at 110° C.; that from A, preparation **20**, weighed 0.8 gram and contained, ash-free, 17.75 per cent. of nitrogen, while that from B, preparation **21**, weighed 0.96 gram and contained 17.61 per cent.

[1] This Journal **13**, 453, 519; **14**, 20. [2] J. Physiology **11**, 435.

Another preparation was made by extracting 25 grams of flax-seed meal with very dilute potash-water, precipitating the proteids by saturation with ammonium sulphate, dissolving the precipitate in brine, dialysing the solution until the globulins had been separated, and concentrating the filtered liquid by boiling to small volume. The coagulum so produced was filtered off, washed with water, alcohol and ether, and dried at 110°. It weighed 0.3 gram, being 1.25 per cent. of the meal. It contained, without correction for ash, 17.65 per cent. of nitrogen—Preparation **22.**

The close agreement in nitrogen between these three preparations indicates a common origin. The proteid precipitated with salt and acid, next to be described, contained about the same proportion of nitrogen and is unquestionably the substance yielding the coagulum on long-continued heating.

2. *Proteids precipitated by Sodium Chloride and Hydrochloric Acid.*

The extract obtained by treating 200 grams of flax-seed meal with 10-per cent. sodium chloride solution, saturating with ammonium sulphate and dialysing the aqueous solution of the precipitate, so obtained, until free from globulin, was treated with sodium chloride until it contained 2 per cent. of the salt. 300 cc. of $\frac{2}{10}$-per cent. hydrochloric acid were then added, and the large precipitate produced filtered off, suspended in 500 cc. of water, and 75 cc. of $\frac{2}{10}$-per cent. hydrochloric acid added. This dissolved a part of the precipitate. The solution was then filtered from the residue, which was washed with alcohol of gradually increased strength up to absolute, then with ether, and finally dried over sulphuric acid. This preparation **23** weighed 6.7 grams and, dried at 110°, contained, ash-free, 17.85 per cent. of nitrogen. Before drying, it was soluble in distilled water, imparting to the solution (which was not coagulated by boiling) a strong acid reaction. Upon neutralising this solution with sodium carbonate, the substance was thrown down in a form insoluble in sodium chloride solution. Addition of salt to the solution gave a precipitate, but nothing separated on adding three volumes of strong alcohol. All these reactions are characteristic of vegetable globulins when dissolved in very dilute acids.

The entire preparation was then dissolved in $\frac{2}{10}$-per cent. hydrochloric acid, and the resulting solution precipitated by carefully

neutralising with sodium carbonate. This reprecipitation was made in order to separate any proteose which might possibly be mixed with the substance. The precipitate was then washed with water, alcohol and ether, and dried at 110°. Its composition was the following:

FLAX-SEED PROTEID.—*Preparation* **23.**

			Ash-free.		
	I.	II.	I.	II.	Average.
Carbon	50.01	50.24	50.03	50.26	50.14
Hydrogen	6.70	6.74	6.70	6.74	6.72
Nitrogen	17.49	17.57	17.50	17.58	17.54
Sulphur } Oxygen }	..	..	..	..	25.70
Ash	0.04	..	..	..	..
					100.00

Ash.—0.4436 gram substance, dried at 110° C., gave 0.0002 gram ash = 0.04 per cent.

Carbon and hydrogen, I.—0.5433 gram dried substance gave 0.9963 gram CO_2 = 50.01 per cent. C, and 0.3277 gram H_2O = 6.70 per cent. H.

II.—0.3428 gram dried substance gave 0.6316 gram CO_2 = 50.24 per cent. C, and 0.2080 gram H_2O = 6.74 per cent. H.

Nitrogen, I.—0.3766 gram dried substance gave 53.15 cc. N at 5° (barometer 766.7 mm. at 20° C.) = 17.49 per cent.

II.—0.3159 gram dried substance gave 45.86 cc. N at 6° (barometer 751.0 mm. at 19° C.) = 17.57 per cent.

Preparation **23**, as just described, after its first precipitation from the dialysed solution by salt and acid, was washed with very dilute hydrochloric acid in which some of it dissolved. These washings were then neutralised with a dilute solution of sodium carbonate, and the precipitate produced filtered off. After washing with water, alcohol and ether, and drying at 110° C., it weighed 1.0 gram—Preparation **24**. Ash-free, it contained 17.80 per cent. of nitrogen.

It is evident that this substance has the same amount of nitrogen as the coagulated proteid derived from similar solutions. To facilitate comparison the analyses are here brought together:

	Prep. **20.**	Prep. **21.**	Prep. **22.**	Prep. **23.**	Prep. **24.**
C	..	..	..	50.14	..
H	..	..	..	6.72	..
N	17.75	17.61	17.65	17.54	17.80

3. *The Proteoses and Peptones.*

The filtrate from preparation **24** was neutralised and then dialysed till free from chlorides, evaporated to very small volume and precipitated with alcohol. After washing with ether and drying over sulphuric acid, the precipitate weighed 1.04 grams —Preparation **25**. This substance had all the properties of a proteose. It dissolved instantly in pure water, was entirely uncoagulable, was precipitated by hydrochloric acid from a solution containing much sodium chloride, and gave a clear pink biuret-reaction. In composition it closely resembles deutero-vitellose, obtained by Chittenden and Hartwell by digestion of crystallised vitellin from the squash-seed with pepsin-hydrochloric acid. Its composition is shown by the following figures, together with that of the deutero-vitellose:

FLAX-SEED PROTEOSE.—*Preparation* **25**.

		Ash-free.	Deutero-vitellose.	
			I.	II.
Carbon	49.08	49.98	50.42	49.27
Hydrogen	6.83	6.95	6.74	6.70
Nitrogen	18.44	18.78	18.43	18.78
Sulphur } Oxygen }	..	24.29	24.41	25.25
Ash	1.80	..	..	..
		100.00	100.00	100.00

Ash.—0.3772 gram substance, dried at 110° C., gave 0.0068 gram ash = 1.80 per cent.

Carbon and hydrogen.—0.3683 gram dried substance gave 0.6628 gram CO_2 = 49.08 per cent. C, and 0.2263 gram H_2O = 6.83 per cent. H.

Nitrogen.—0.3500 gram dried substance gave 52.35 cc. N at 11.5° C. (barometer 780.4 mm. at 22° C.) = 18.44 per cent.

The filtrate from preparation **23**, which had been obtained by adding 2 per cent. of sodium chloride and some hydrochloric acid to the dialysed extract freed from the globulins, was next saturated with sodium chloride and a small precipitate obtained. This was filtered off, dissolved in water, the solution dialysed until free from chlorides, evaporated to small volume, precipitated with alcohol, washed with ether and dried over sulphuric acid. 0.80 gram of proteose was thus obtained. When dried at 110° it contained, ash-free, 18.33 per cent. of nitrogen—Preparation **26**.

The solutions freed from globulin by dialysis and from coagulable bodies by concentration, were in all cases found to contain a considerable amount of proteid matter having the properties of a mixture of proteoses and peptones. This mixed substance was very readily soluble in water to a solution which, when saturated with ammonium sulphate while hot, and filtered, gave a strong red biuret-reaction, and no precipitate with copper sulphate showing the presence of peptones. This was observed in all of the five preparations examined. The aqueous solution of the substance was precipitated by copper sulphate, gave a precipitate with nitric acid which dissolved on heating and reappeared on cooling, and a precipitate with ammonium sulphate. These reactions show the presence of proteoses.

The Proteids insoluble in Water and Sodium Chloride Solutions.

After complete extraction with sodium chloride solution, more or less proteid was always found in the residue of the meal, most of which could be removed by treatment with $\frac{2}{10}$-per cent. potash solution. The amount of this substance was not large, in three cases being 1.30, 1.55 and 0.66 per cent., respectively. In no case were preparations obtained which appeared to be pure. The alkali extracted from the outer coating of the seed a large amount of coloring matter which on neutralisation was always precipitated with the proteid, imparting to the preparations a chocolate-brown color. The large amount of gum contained in the seed also rendered the preparation of these substances very difficult, as this gum was much more soluble in the alkaline liquid than in salt or acid solutions, and on neutralisation was undoubtedly carried down with the proteid. As a result no definite composition could be assigned to the substance so extracted. This result might be expected, even if the difficulties just mentioned did not exist, were the proteid substance still contained in the seed different in composition from the globulin; for this latter would necessarily be but imperfectly extracted by salt solutions, and would be taken up by the potash and precipitated on neutralisation. For these reasons the large number of preparations made and analysed gave very varying results and afforded no data for even an approximate statement of the composition of the proteid extracted. After complete

exhaustion with $\frac{2}{10}$-per cent. potash solution, the extracted residue always contained a considerable amount of nitrogen, which may have escaped solution through protection afforded by the tissue of the seed, or it may have belonged to bodies insoluble in the reagents employed. No evidence on this point was obtained.

VI.—The Amount of the various Proteids separated from the Flax-seed.

The results of this investigation, thus far recorded, show that the extracts of the flax-seed contain a globulin precipitable by dialysis; a proteid, resembling both globulin and albumin, precipitable by long-continued heating at 100°, as well as by sodium chloride in the presence of acid; proteose and peptone-like bodies, and a proteid not extracted by sodium chloride solution, but soluble in dilute potash-water.

All attempts to determine the amount of these various substances failed because of change, while in solution, into non-proteid bodies. It was further found that the relative amount of the various proteids was very variable; it is almost certain that the more soluble forms were largely, if not wholly, derived from the globulin in consequence of alteration during extraction aud separation. These operations were in all cases greatly prolonged on account of the gum contained in the seed, which rendered filtration extremely difficult and slow. Putrefaction, however, was entirely prevented by the use of thymol. Numerous attempts were made to determine the total proteid-nitrogen in the seed, but in no case did the nitrogen extracted and recovered in the proteid precipitates, added to that in the extracted residue, equal the total nitrogen contained in the flax-seed meal.

This may be illustrated by the following figures: Two portions of 100 grams of flax-seed meal containing 8.40 per cent. of nitrogen were extracted,—A, first with water, then with 10-per cent. sodium chloride solution and, finally, with $\frac{2}{10}$-per cent. potash-water. B, first with 10-per cent. sodium chloride solution and then with $\frac{2}{10}$ per cent. potash-water. The amounts of proteid obtained, together with the nitrogen of each, were as follows, in per cent. of the meal:

	A.		B.	
	Proteid.	Nitrogen.	Proteid.	Nitrogen.
Total globulin	13.5	2.5139	16.05	2.9206
Potash extracted	1.3	0.1808	1.55	0.2305
Coagulum	0.8	0.1406	0.96	0.1674
Proteose	3.7	0.5872	1.30	0.1648
Total	19.3	3.4225	19.86	3.4833
Nitrogen in insoluble residue		0.4255		0.4556
Total accounted for		3.8480		3.9388
Nitrogen lost		4.5520		4.4612
		8.4000		8.4000

These two extractions were made at the same time and under the same conditions, and the results are in striking agreement. In extraction A, 0.4255 gram of nitrogen out of 8.4 grams of total nitrogen remained in the residue of the seed, showing that 94.94 per cent. of the total nitrogen was soluble in the reagents employed. The nitrogen extracted by potash-water amounted to 2.15 per cent. of the total, so that by water and by sodium chloride solution 92.79 per cent. of the total nitrogen was extracted. The nitrogen recovered from these solutions amounted to 38.59 per cent. of the total, so that the nitrogen lost equaled 4.5528 grams, or 54.2 per cent. This loss must have been due either to the presence of non-proteid nitrogenous substances, or to a decomposition of the proteid matter into diffusible nitrogen-containing compounds.

Numerous attempts were made to determine the total amount of proteid-nitrogen, with, however, very varying results. In one case 20 grams of flax-seed meal (=16.74 grams dried at 110° C.) were extracted with $\frac{2}{10}$-per cent. potash solution, the extract filtered, and neutralised with $\frac{2}{10}$-per cent. hydrochloric acid. The neutralisation-precipitate was filtered off, washed with water, alcohol and ether and dried at 110°. It weighed 5.4815 grams (=32.74 per cent. of the meal taken), and contained 0.9176 gram nitrogen—equivalent to 63.44 per cent. of the total nitrogen of the meal. The insoluble residue of the meal contained 0.2486 gram nitrogen=17.19 per cent. The nitrogen accounted for was in this case 80.53 per cent. of the total.

In another experiment 50 grams of flax-seed meal were extracted with 1000 cc. of 20-per cent. brine and filtered as rapidly as possible. 500 cc. of the extract (=25 grams of the flax-seed meal, or

20.925 grams dried at 110° C.) were then precipitated as completely as possible with dilute hydrochloric acid, and the resulting precipitate filtered off and washed with a dilute solution of sodium chloride containing a very little hydrochloric acid, then with alcohol and ether. Dried at 110° it weighed 5.7555 grams. This precipitate was therefore 27.50 per cent. of the meal taken and contained 0.8927 gram nitrogen=49.38 per cent. of the total nitrogen of the meal. The filtrate from this preparation was examined carefully for precipitable proteids, but no more could be obtained. After neutralising, the solution and washings were put in a dialyser and the salts separated. The solution was then evaporated to small volume and precipitated with absolute alcohol; the precipitate was washed with ether and dried at 110°. During the concentration a little coagulum was found, but this was not filtered off. In this way 2.36 grams of substance were obtained, having a large amount of ash. Ash-free, this substance formed 9.61 per cent. of the meal employed and contained 0.2294 gram of nitrogen=12.69 per cent. of the total nitrogen. The amount of nitrogen in these two preparations was 62.07 per cent. of the total. The nitrogen remaining in the meal after extraction was not determined on account of the very difficult filtration caused by the gum.

If this nitrogen is assumed to be the same as that contained in the residue extracted by potash, *i. e.* 17.19 per cent. of the total nitrogen, as described in the preceding experiment, the nitrogen accounted for would be 79.26 per cent. of the total. If we assume that in the first experiment the proteose present was equal to that found in the second, we should then account for 93.22 per cent. of the nitrogen. These experiments therefore indicate that the loss of nitrogen observed is due to a change in the proteids resulting in diffusible nitrogenous products.

To obtain further evidence on this point the seed was most carefully examined for other nitrogenous substances according to E. Schulze's method, but with negative results. It was, however, found that much non-proteid nitrogen occurred in the diffusate, and that the amount of nitrogen passing out of the dialyser in equal times was tolerably constant and therefore unquestionably due to a slow and uniform decomposition. This is shown by the following experiments:

100 grams of flax-seed meal suspended in 1500 cc. of distilled water were placed in a dialyser immersed in an equal volume of

distilled water. After 48 hours the diffusate was carefully tested without finding any proteid matter. The solution was then acidified with pure sulphuric acid, concentrated to small volume and the nitrogen present determined by Kjeldahl's method to be 0.1808 gram. This process was repeated six times, and at the conclusion of the experiment the contents of the dialyser were found to be wholly free from any odor or other evidence of putrefaction, having been protected by a liberal use of thymol. The amount of nitrogen found to have passed the parchment after each diffusion-period of 48 hours was as follows:

1st	diffusate	0.1808 gram.
2d	"	0.3077
3d	"	0.2577
4th	"	0.3445
5th	"	0.3417
6th	"	0.3042
Total nitrogen		1.7366

The fact that the nitrogen contained in the first diffusate was less than that in the others, and that the latter contained a nearly constant amount, is strong evidence that the diffusible nitrogenous substances did not exist ready-formed in the seed at the beginning of the experiment, but resulted from a slow decomposition of the proteid.

Several other trials of this character gave the same result, the diffusate in all cases being free from proteid matter, but containing bodies which evolved ammonia on heating with caustic alkalies. The nature of these substances was not discovered. It might naturally be supposed that this change was brought about through the action of a ferment similar to that contained in the pawpaw and the pineapple, but careful experiments with acid, alkaline and neutral extracts of the flax-seed proved it to be wholly free from any ferment capable of digesting coagulated egg-albumin, even after many hours contact at 40°.

Extraction of the flax-seed with water at 40° for some hours and subsequent exhaustion with 10-per cent. sodium chloride solution, gave no evidence of a proteolytic ferment, the yield of globulin under this treatment being unusually large. Extraction with a 20-per cent. solution of sodium chloride heated to 70°—whereby it was thought a ferment might be coagulated and rendered inoperative—yielded no more globulin than usual: in fact all attempts

failed to disclose satisfactory evidence of the existence of a ferment.

It is to be noted that when the flax-seed meal was directly extracted with dilute potash-water, 32.74 per cent. of the meal was obtained in the form of a precipitated proteid when the solution was neutralised. The insoluble residue of the meal further yielded 0.2486 gram of nitrogen, which would be equivalent to 8.0 per cent. of proteid containing the amount of nitrogen found in the globulin, *viz.*, 18.6 per cent. The nitrogen of the neutralisation precipitate would be equivalent to 29.4 per cent. of globulin. The sum of these would be 37.4 per cent. of the meal.

The results of this investigation already given show that the globulin and the body extracted by dilute potash are the only proteids found in the extract which are precipitated from an alkaline solution on neutralisation. Therefore these two proteids must be the only ones contained in the precipitate. If we assume that the proteids in the flax-seed have, on the average, 18 per cent. of nitrogen, the meal extracted in this case would contain 48.0 per cent. of proteids. The 37.4 per cent. thus accounted for amounts to 78.0 per cent. of the total proteids.

In extract A (see page 656), by the same method of calculation, —*i. e.*, adding together the nitrogen of the insoluble residue, that of the potash-extract and of the globulin, and multiplying by 5.37 (the nitrogen-factor for a substance containing 18.6 per cent. of nitrogen)—we account for 16.75 per cent. of the meal, or only 36.3 per cent. of the total proteids. The difference between 78 per cent. (found in the potash extract) and 36.3 per cent. (in extract A) is 41.7 per cent., which loss must clearly have been caused by a breaking up of the globulin or of the body extracted by potash-water. It has been shown (page 656) that in extract A 92.79 per cent. of the nitrogen was extracted by salt solution and water, and that more than two-thirds of the remaining 7.21 per cent. of nitrogen was contained in the residue and did not go into solution. It is clear, therefore, that the substance extracted by potash solution cannot be considered as the source of the loss. It is therefore certain that the globulin is the principal proteid suffering change.

As a confirmation of the idea that the crystallisable globulin suffers this loss, and also yields a large proportion of the water-soluble proteids, the results obtained by dialysing salt solutions of the globulin are important. 10 grams of air-dry crystallised

globulin were dissolved in 10-per cent. sodium chloride solution and the clear filtered liquid placed in a dialyser. After complete removal of the chlorides the deposited globulin was filtered off, and the filtrate found to give a strong biuret-reaction and a precipitate with sodium chloride and acid.

In another case 17 grams of air-dry crystalline globulin were dissolved in 10-per cent. sodium chloride solution, the insoluble matter was filtered off and, after thorough washing with salt solution, water, alcohol and ether, was found to weigh 1.27 grams. The clear solution was dialysed free from chlorides in water of 6°, and filtered from the separated globulin. This, after washing with water, alcohol and ether, weighed, air-dry, 12.19 grams. The clear filtrate gave a biuret-reaction of a decided red tint, and on adding hydrochloric acid to its solution, saturated with sodium chloride, a precipitate. This solution was then concentrated and 0.11 gram of coagulum obtained. Filtered from this coagulum and precipitated with alcohol the solution gave 0.4 gram of proteose. It is thus evident that the salt solution of the globulin yields substances of the same character as those obtained from the extract of the seed. The percentage amount of these substances is shown by the following figures:

Globulin taken	17.00 grams	
Insoluble	1.27	
Dissolved globulin	15.73 grams	
Recovered "	13.46	85.460 per cent.
" coagulum	0.11	0.007
" proteose	0.40	0.028
Loss		14.505
		100.000

It is to be noted that these figures show a loss of 14.5 per cent. of proteid matter. This is far in excess of any mechanical loss which could have occurred, and can only be explained by a change in the proteid whereby diffusible substances were produced and, perhaps, also others not precipitable by strong alcohol. In either case substances other than proteid were formed. A similar loss of proteids was observed in all the extractions.

It has been shown that after separating the globulin as completely as possible by dialysis, there was found in the solution a body similar to albumin but having properties in common with globulin. The amount of this substance varied greatly, in some

cases very little being found and in others large amounts. This was, unquestionably, for the most part, if not wholly, derived from the globulin, for in those extracts in which it occurred in the largest amount the globulin was found in greatly diminished quantity. A proteid having similar reactions was also found in the aqueous solution obtained by redissolving the globulin in sodium chloride brine and separating a second time by dialysis. Whether this substance exists in the seed in small quantities, or is wholly derived from the globulin during the process of extraction and separation, cannot be determined from the results of the present investigations.

In regard to the amount of proteoses and peptones the same must be said. It is pretty certain that the peptones are wholly formed during the extraction and separation, as no evidence of their presence in the extract when first made could be obtained. It is not possible, however, to detect minute quantities of peptones with certainty in strongly colored vegetable extracts rich in sugars.

It is exceedingly desirable to determine as accurately as possible the amount of each proteid contained in the seed, and to know exactly its nitrogen-content so that an accurate factor may be obtained for calculating the proportion of proteids from the nitrogen found.

It is evident from the preceding statements that it is impossible to say exactly what this factor should be for flax-seed, but a pretty close approximation can be made—one at least far more accurate than the factor at present in use based on a content of 16 per cent. of nitrogen. It has been shown on page 656 that about 93 per cent. of the nitrogen of the seed is extracted by salt solutions. This nitrogen has been shown to belong chiefly to the globulin which contains 18.6 per cent. of nitrogen. The albumin-like body was found to contain 17.7 per cent. of nitrogen, and the proteose prepared in a pure state contained in one case 18.78 per cent. and in another 18.33 per cent. of nitrogen. If, then, we assume the proteids to contain collectively an average of 18.00 per cent. of nitrogen we will not be very far from the truth. The factor then for flax-seed would be 5.55 instead of 6.25, and, for a sample of linseed meal containing 7 per cent. of nitrogen, would give a content of proteid of 38.85 per cent. instead of 43.75—a difference of very considerable magnitude.

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CRYSTALLISED VEGETABLE PROTEIDS.

By Thomas B. Osborne.

The existence of crystallised proteids in seeds was pointed out by Hartig in 1855. Four years later, Maschke obtained hexagonal plates of proteid matter by extracting Brazil-nuts with water heated to 40°–50° and evaporating the filtered extract at 40°. Nageli[1] investigated the crystal-like forms from the Brazil-nut as well as the artificially produced crystals of Maschke and concluded that they differed in some respects from true crystals. He therefore designated them as "crystalloids."

Sachsse,[2] following Maschke's method, and also by precipitating the aqueous extract with carbonic acid, obtained several preparations of proteid from the Brazil-nut which he analysed. These preparations were, however, not distinct crystals, but were composed of small discs.

Schmiedeberg[3] obtained crytallised products by treating the aqueous extract of the Brazil-nut with carbon dioxide, washing the precipitated proteid, digesting the precipitate with magnesia suspended in water at 35°, filtering and evaporating at the same temperature. These crystals he considered to be a compound of the proteid with magnesia.

Drechsel[4] obtained crystals, presumably hexagonal plates, by submitting the solution containing the "magnesia compound," prepared as Schmiedeberg suggested, to dialysis in a vessel containing alcohol.

Drechsel afterwards obtained finely developed crystals by allowing a warm sodium chloride solution saturated with the proteid of the Brazil-nut to cool slowly, when the substance separated in well-formed crystals.[5]

At Drechsel's suggestion Grübler applied this method with some modifications to the proteids of squash-seed, and made a large number of preparations of perfectly formed octahedral crystals whose properties and composition he described at length.[6]

[1] Botanische Mittheilungen (München, 1863), Vol. I.
[2] Die Farbstoffe. Kohlenhydrate und Proteinsubstance (Leipzig, 1877), p. 315.
[3] Ztschr. physiol. Chem. **1**, 205.
[4] J. prakt. Chem. **19**, 331.
[5] Grübler: *cf. ibid.* **23**, 100.
[6] J. prakt. Chem. **23**, 97.

Ritthausen[1] obtained crystallised preparations from the castor-bean, hemp-seed and sesame-seed, which separated from a warm sodium chloride solution on cooling in the form of regular octahedrons. The composition and properties of the crystals obtained from the hemp-seed and castor-bean he described in a subsequent paper.[2]

The writer next prepared rhombohedral and octahedral crystals from the oat-kernel by cooling a warm dilute sodium chloride solution saturated with the proteid.[3]

The writer has also obtained octahedral crystals from flax-seed by extracting with a solution of sodium chloride and dialysing the filtered extract; the proteid separating in well-formed crystals as the salts were removed.[4]

The fact that these proteid substances can be artificially crystallised is not only interesting in itself, but is important as presumably furnishing a means for making preparations of undoubted purity which will afford a sure basis for further study of their properties. The contradictory statements made by the various investigators, not only in regard to properties and composition of these bodies but also in respect to the value of the methods of solution and separation which have been employed hitherto, render an exact knowledge of all the facts relating to these substances a matter of the highest scientific and practical importance.

An examination of the literature indicates that definitely crystallised preparations from the Brazil-nut have never been analysed, and also shows that the published analyses do not agree sufficiently to fix the composition of the substance.

Ritthausen's observations on the properties of the crystallised proteids of the hemp-seed and castor-bean indicate that they are closely related to, if not identical with, the body obtained by Grübler from the squash-seed; yet the results of Ritthausen's analyses differ widely from those first made by Grübler.

The composition of the crystallised proteid of the squash-seed has been fixed within narrower limits by the analyses of Chittenden and Hartwell,[5] Ritthausen,[6] and Grübler.[7] It seemed desirable, therefore, that the composition and properties of the

[1] J. prakt. Chem. **23**, 481.
[2] *Ibid.* **25**, 130.
[3] Report Conn. Agri. Expt. Station 1890, and This Journal **13**, 408; **14**, 212.
[4] This Journal **14**, 329, 629.
[5] J. of Physiology **11**, 435.
[6] J. prakt. Chem. **25**, 137.
[7] *Ibid.* **44**, 369.

various crystallised proteids should be investigated anew in order to determine the relations of these substances.

As the writer had already prepared crystallised products from the oat-kernel and flax-seed, he determined to reinvestigate the similar bodies obtainable from the Brazil-nut, hemp-seed, castor-bean and squash-seed, and the results of this investigation are embodied in the following pages.

Crystallised Proteid of the Brazil-nut, *Bertholletia.*

If a thin section of the meat of a Brazil-nut is examined under the microscope, after washing with ether and then with alcohol, a large number of bodies may be seen which more or less resemble crystals in form. If cold water is next applied to the section, an outer coating of these masses appears to be dissolved and a great number of apparently rhombohedral crystals are set free. These crystals are proteid in character, and on adding some sodium chloride solution dissolve instantly. If warm water is used instead of cold, the substance of these crystals dissolves, doubtless in the dilute saline solution which is formed with the alkali-salts of the seed, but which, when cold, has little, if any, solvent power. This view is supported by the fact that the proteid extracted from the seed is wholly insoluble in pure water, either cold or warmed to 40°.

Maschke,[1] as already stated, made a solution of this substance by extracting the oil-free meats with water and evaporating at 40°–50°. He thus obtained thick six-sided plates which he says "belong undoubtedly to the regular system, and the same form would be obtained if an octahedron were cut into three equal parts parallel to one of its octahedral faces; the middle part would then produce in every respect one of these six-sided plates."

Maschke made an analysis of his preparations, but considered that the substance was a compound of casein with an acid. Sachsse,[2] following Maschke's directions, failed to obtain crystals, the substance separating in small discs (*scheibchen*). He also obtained small discs by precipitating the aqueous extract with carbonic acid.

As a mean of five analyses of the preparations made after drying at 100°–110°, he gave the following:

[1] J. prakt. Chem. **74**, 436.

[2] Die Farbstoffe, Kohlenhydrate und Proteinsubstance (Leipzig, 1877), p. 315.

CRYSTALLISED GLOBULIN OF BRAZIL-NUT.

BRAZIL-NUT GLOBULIN.—*Sachsse.*

	a. discs.
Carbon	51.00
Hydrogen	7.25
Nitrogen	18.06
Sulphur	1.36
Phosphoric acid (P_2O_5)	0.82
Oxygen	21.51
	100.00
Ash	0.76

The ash was determined by burning the substance mixed with calcium phosphate, and was found to equal in amount the phosphoric acid obtained by fusing with soda and potassium nitrate and precipitating with uranium acetate. Sachsse therefore concluded that the substance belonged to the class of vegetable caseins.

Th. Weyl,[1] in a paper on animal and vegetable proteids, published an analysis of the proteid of the Brazil-nut obtained by extracting the oil-free meats with 10-per cent. sodium chloride solution, precipitating by diluting and passing through the solution carbonic acid gas, washing the precipitate with water and then boiling with alcohol and drying.

He found the preparation thus made, after deducting the ash, to have the following composition:

BRAZIL-NUT GLOBULIN.—*Weyl.*

	b.
Carbon	52.43
Hydrogen	7.12
Nitrogen	18.10
Sulphur	0.55
Oxygen	21.80
	100.00

Weyl considered the substance to be a globulin resembling the vitellin obtained from the yolk of eggs.

Ritthausen,[2] replying to the criticisms of Weyl on the methods employed by him which involved the use of weak alkali solutions in preparing proteids from seeds, published the results obtained by extracting the oil-free meats of the Brazil-nut with a dilute

[1] Ztschr. physiol. Chem. **1**, 72. [2] Pflüger's Archiv **16**, 301.

solution of potash, precipitating the filtered extract with acetic acid, and washing the precipitate so produced with water, alcohol and ether. After drying at 130°, Ritthausen found his preparation to have the following composition:

BRAZIL-NUT GLOBULIN.—*Ritthausen.*

	c.
Carbon	52.29
Hydrogen	7.24
Nitrogen	18.09
Sulphur	1.32
Oxygen	21.06
	100.00

The considerable discrepancies among the foregoing analyses and the fact that none of the preparations were distinctly crystallised make further investigations desirable. For this purpose a large quantity of fresh Brazil-nuts was procured, the outer shells removed, the perfectly sound meats were carefully selected, chopped fine and treated with benzine (petroleum-ether) in order to remove the oil. The finer portions of the material were then washed through a straining cloth by the aid of benzine, and a quantity of fine white meal was thus obtained.

100 grams of this meal were then treated with 10-per cent. sodium chloride solution and the very slightly colored extract filtered perfectly clear. The residue of the meal was treated twice more in the same way with sodium chloride solution, and the extracts obtained were united and saturated with pure ammonium sulphate. The snow-white precipitate resulting was thrown on a filter and, when freed from the solution, was removed from the paper and suspended in distilled water. The dilute solution of ammonium sulphate thus produced dissolved but a part of the precipitate. Complete solution resulted immediately on adding a little sodium chloride, showing that this latter salt is a far more energetic solvent for this substance than ammonium sulphate. The solution, after filtering perfectly clear, was placed in a dialyser and suspended in running water for three days, when it was removed and filtered from the large amount of separated substance. The solid product was washed thoroughly with water, alcohol and ether, and dried over sulphuric acid. It weighed nearly 25 grams. Under the microscope it was seen to consist wholly of crystals in the form of hexagonal plates, as shown in the accompanying plate. Dried at 110° these crystals had the following composition:

BRAZIL-NUT GLOBULIN: *Crystals—Preparation* **1.**

	I.	II	Average.	Ash-free.
Carbon	52.11	52.05	52.08	52.18
Hydrogen	6.91	6.92	6.92	6.92
Nitrogen	18.28	18.25	18.27	18.30
Sulphur	1.03	1.06	1.05	1.05
Oxygen	..	..	..	21.55
Ash	0.20	..	..	..
				100.00

Ash.—0.8475 gram substance, dried at 110°, gave 0.0017 gram ash=0.20 per cent.

Carbon and hydrogen, I.—0.3702 gram dried substance gave 0.7072 gram CO_2=52.11 per cent. C, and 0.2303 gram H_2O=6.91 per cent. H.

II.—0.3736 gram dried substance gave 0.7130 gram CO_2=52.05 per cent. C, and 0.2326 gram H_2O=6.92 per cent. H.

Nitrogen, I.—0.3887 gram dried substance gave 57.14 cc. N at 5° (barometer 769.5 mm. at 22°) = 18.28 per cent.

II.—0.3953 gram dried substance gave 57.74 cc. N at 5° (barometer 773.0 mm. at 22°) = 18.25 per cent.

Sulphur, I.—0.7940 gram dried substance gave 0.0596 gram $BaSO_4$ = 1.03 per cent. S.

II.—0.6020 gram dried substance gave 0.0465 gram $BaSO_4$ = 1.06 per cent. S.

A second preparation of this substance was made by extracting 50 grams of the oil-free meats with 10-per cent. sodium chloride solution and at once dialysing the clear filtered extract. During two days nothing solid appeared in the dialyser, but soon after a deposit began to form, and at the end of seven days a considerable precipitate had separated which was filtered off, washed with water, alcohol and ether, and when dried over sulphuric acid weighed 6 grams. Under the microscope this substance appeared to consist of small spheroids with a more or less irregular outline, strongly suggesting crystals, but presenting no definite crystalline form. Dried at 110°, the substance yielded the following results on analysis:

BRAZIL-NUT GLOBULIN: *Spheroids—Preparation* **2.**

		Ash-free.
Carbon	51.87	52.35
Hydrogen	6.90	6.96
Nitrogen	18.00	18.16
Sulphur	1.11	1.12
Oxygen	..	21.41
Ash	0.92	..
		100.00

Ash.—0.6811 gram substance, dried at 110°, gave 0.0063 gram ash = 0.92 per cent.

Carbon and hydrogen.—0.2919 gram dried substance gave 0.5515 gram CO_2 = 51.87 per cent. C, and 0.1823 gram H_2O = 6.90 per cent. H.

Nitrogen.—0.3963 gram dried substance gave 57.14 cc. N at 5° (barometer 772.7 mm. at 23°) = 18.00 per cent.

Sulphur.—0.8100 gram dried substance gave 0.0648 gram $BaSO_4$ = 1.11 per cent. S.

A third preparation was made by extracting 25 grams of the oil-free meal with distilled water heated to 60°. The solution was then allowed to stand several days in a cold room at a temperature of about 5°, thymol being freely used to prevent decomposition. After the separated substance had settled, the supernatant solution was decanted, the sediment collected on a filter, washed with water, alcohol and ether, and dried over sulphuric acid. It weighed 7 grams. The particles composing this precipitate were similar to those of the preparation last described, but smaller. Dried at 110°, it was found by analysis to have the following composition:

BRAZIL-NUT GLOBULIN: *Spheroids—Preparation* 3.

		Ash-free.
Carbon	51.78	52.16
Hydrogen	6.93	6.98
Nitrogen	18.19	18.32
Sulphur	1.06	1.07
Oxygen	..	21.47
Ash	0.72	..
		100.00

Ash.—0.7667 gram substance, dried at 110°, gave 0.0055 gram ash = 0.72 per cent.

Carbon and hydrogen.—0.3252 gram dried substance gave 0.6174 gram CO_2 = 51.78 per cent. C, and 0.2030 gram H_2O = 6.93 per cent. H.

Nitrogen.—0.4062 gram dried substance gave 59.42 cc. N at 5° (barometer 769.5 mm. at 22°) = 18.19 per cent.

Sulphur.—0.8210 gram dried substance gave 0.0630 gram $BaSO_4$ = 1.06 per cent. S.

These analyses show that this proteid has the same composition whether crystallised or spheroidal, and that the different methods of extraction give the same results. To facilitate comparison, all the analyses are here tabulated.

BRAZIL-NUT GLOBULIN.

	Sachsse.[1] *a* discs.	Ritthausen. *b*	Weyl. *c*	Osborne. 1 crystals.	Osborne. 2 spheroids.	Osborne. 3 spheroids.
Carbon	51.42	52.29	52.43	52.18	52.35	52.16
Hydrogen	7.31	7.24	7.12	6.92	6.96	6.98
Nitrogen	18.21	18.09	18.10	18.30	18.16	18.32
Sulphur	1.37	1.32	0.55	1.06	1.12	1.07
Oxygen	21.69	21.06	21.80	21.54	21.41	21.47
	100.00	100.00	100.00	100.00	100.00	100.00

With the exception of Sachsse's carbon determination, the figures for this element and for nitrogen are in fair agreement. The figures for hydrogen obtained by the writer are somewhat lower than the others, but as special precautions were taken to keep the reagents absolutely free from moisture, and as these agree with each other, it is probable that they are more nearly correct.

Weyl's sulphur determination is unquestionably incorrect. Sachsse and Ritthausen obtained about 0.2 per cent. more sulphur than the writer, but as it does not appear that either of these investigators examined the ash of their preparations for sulphate, it is possible that the cause of this difference may be due to sulphur present in such form. The writer's reagents were absolutely free from any trace of sulphur.

In view of these facts, and also since preparation 1 was well crystallised and in all probability pure, we may fairly take its analysis as closely representing the composition of the crystalline proteid of the Brazil-nut.

There can be little doubt that this artificially crystallised proteid is identical with that naturally occurring in the Brazil-nut, with which it agrees in its properties. The fact that it crystallises during dialysis is strong evidence that it is the same substance, entirely unchanged, for the "crystalloids" of the seed almost certainly were deposited in its cells by a similar process.

It is true that the artificial crystals are hexagonal plates, while the majority of the natural crystals resemble rhombohedrons, but the writer has shown that oat-globulin under a slight change of conditions may yield either octahedral or rhombohedral crystals. So the proteid of hemp-seed sometimes gives hexagonal plates, although as a rule octahedral crystals are obtained.

[1] Sachsse's analysis is here calculated free from phosphoric acid.

The reactions of the artificially crystallised proteid of the Brazil-nut are as follows:

Insoluble in pure distilled water, even on warming to 50° C. Soluble in solutions of sodium chloride, from which it is partly precipitated on dilution with water, but not thrown down by saturation with sodium chloride. Precipitated completely by saturation with ammonium sulphate, and partially by saturation with magnesium sulphate. Very soluble in distilled water containing a minute amount of acid.

Water containing $\frac{2}{100}$ per cent. of hydrochloric acid dissolves the proteid at once; the amount of acid required being proportional to the quantity of substance. In other words, sufficient acid must be present to convert all the substance into an acid compound before complete solution results. Addition of a very small amount of sodium chloride, or other salt, precipitates the solution. This precipitate is insoluble in a large amount of sodium chloride, denoting the formation of an acid-compound. These acid solutions do not coagulate on boiling and are not precipitated by alcohol.

Citric acid dissolves the proteid less readily than hydrochloric acid, solutions of $\frac{1}{20}$ per cent. having but little solvent power. $\frac{1}{2}$ per cent. solution of citric acid gives a turbid solution, which becomes clear at once on addition of a little sodium chloride, but is precipitated on adding more of the salt, the precipitate being insoluble in an excess.

Dilute sodium carbonate solutions of $\frac{1}{2}$ to $\frac{1}{4}$ per cent. dissolve the proteid instantly and completely, as do also solutions containing a very little caustic alkali. On careful neutralisation the proteid is thrown down unchanged, being readily and completely soluble in sodium chloride solution. If acid is added in excess the proteid dissolves, and on again neutralising is precipitated in a form soluble in sodium chloride.

Dissolved in 10-per cent. sodium chloride solution a turbidity is produced on gradually heating to 70°, and on raising the heat to 86° flocks separate, which increase in amount if the temperature is slowly raised to 100°. This substance responds to all the usual tests for proteids, such as the biuret, Millon's, xanthoproteic, etc. The same reactions were given by the preparations made by cooling the warm aqueous extract. These reactions show this proteid to be a true globulin of the vitellin class.

Crystallised Proteid of Hemp-seed.

As already stated, Ritthausen has described[1] a crystalline preparation obtained by extracting powdered hemp-cake with 5-per cent. sodium chloride solution heated to 40°, and allowing the filtered extract to cool. The proteid separated in isometric crystals. The filtrate on diluting gave a further precipitate, which was united with the first and recrystallised according to the method used by Grübler in his work on the squash-seed. A perfectly crystallised product was thus obtained, consisting of "octahedra, rhombodedecahedra and other forms of the regular system."

In a subsequent paper[2] Ritthausen published the results of his analyses of this proteid. Four preparations were made and analysed, only one of which, however, was crystallised. These were obtained: *d*, by diluting the sodium-chloride extract with water; *e*, by extracting *d* with 20-per cent. sodium chloride solution and preparing the insoluble portion for analysis; *f*, by crystallising *d* by Drechsel's method; *g*, by extracting the seeds with $\frac{1}{10}$-per cent. potash solution. The composition of these products was as follows:

	Ritthausen.			
	d.	*e.*	*f.* Crystals.	*g.*
Carbon	50.94	51.33	50.98	51.19
Hydrogen	6.85	6.84	6.92	6.97
Nitrogen	18.62	18.80	18.73	18.06
Sulphur	0.8	0.82	0.82	0.92
Oxygen	22.74	22.21	22.55	22.86
	100.00	100.00	100.00	100.00

These analyses agreed with the results first obtained by Grübler for the proteid of the squash-seed, with the exception of carbon, of which Grübler found 53.30 per cent. Ritthausen[3] then procured some of Grübler's preparation and redetermined the carbon, finding as an average of four analyses 51.61 per cent. This result has since been confirmed by Grübler himself,[4] who now gives the percentage of carbon as 51.48 per cent.

It will be noticed that the carbon of Ritthausen's crystallised preparation is 0.5 per cent. lower than these figures and throws some doubt on the identity of these substances. In order to clear

[1] J. prakt. Chem. **23**, 481. [2] *Ibid.* **25**, 130. [3] *Ibid.* **25**, 137 [4] *Ibid.* **44**, 369.

up this point, 100 grams of ground hemp-seeds—from which the oil had been removed by extracting with benzine, and the greater part of the hulls separated by passing through a fine sieve—were extracted with 10-per cent. sodium chloride solution and the filtered extract placed in a dialyser set in running water. After five days a large deposit was found in the dialyser, consisting almost wholly of octahedral crystals mixed with a few spheroids. This substance was filtered off, washed with water, alcohol and ether and dried over sulphuric acid. 7.8 grams were so obtained, which, when dried at 110°, had the following composition:

HEMP-SEED GLOBULIN: *Crystals—Preparation* **4.**

		Ash-free.
Carbon	51.00	51.26
Hydrogen	6.83	6.86
Nitrogen	18.59	18.68
Sulphur	0.94	0.94
Oxygen	..	22.26
Ash	0.50	..
		100.00

Ash.—0.4042 gram substance, dried at 110°, gave 0.0020 gram ash = 0.50 per cent.

Carbon and hydrogen.—0.2977 gram dried substance gave 0.5567 gram CO_2 = 51.00 per cent. C, and 0.1840 gram H_2O = 6.83 per cent. H.

Nitrogen.—0.3464 gram dried substance gave 53.96 cc. N at 6° (barometer 741.7 mm. at 22°) = 18.59 per cent.

Sulphur.—0.9930 gram dried substance gave 0.0670 gram $BaSO_4$ = 0.92 per cent. S.

Another preparation was made by extracting 200 grams of ground hemp-seeds, freed from oil, with two liters of 5-per cent. sodium chloride solution heated to 60°, and filtering while hot. On cooling gradually to 5° a large amount of substance separated, which consisted wholly of octahedral crystals. This substance was then filtered off and washed with water. After most of the salt had been washed out, the preparation began to dissolve, the washings being precipitated on falling into the filtrate containing sodium chloride. Dilute alcohol was then substituted for water and the washing continued, the alcohol being gradually increased in strength to absolute. After washing next with ether the preparation was dried over sulphuric acid, and weighed 12.25 grams. Dried at 110° it had the following composition:

HEMP-SEED GLOBULIN: *Crystals—Preparation* **5**.

		Ash-free.
Carbon	51.24	51.28
Hydrogen	6.84	6.84
Nitrogen	18.83	18.84
Sulphur	0.87	0 87
Oxygen	..	22.17
Ash	0.07	..
		100.00

Ash.—0.7188 gram substance, dried at 110°, gave 0.0005 gram ash = 0.07 per cent.

Carbon and hydrogen.—0.4022 gram dried substance gave 0.7556 gram CO_2 = 51.24 per cent. C, and 0.2477 gram H_2O = 6.84 per cent. H.

Nitrogen.—0.3495 gram dried substance gave 54.16 cc. N at 6° (barometer 754.7 mm. at 23°) = 18.83 per cent.

Sulphur.—0.9355 gram dried substance gave 0.0597 gram $BaSO_4$ = 0.87 per cent. S.

A small amount of substance similarly prepared was found to contain, dried at 110°, 18.72 per cent N and 0.30 of ash, or, ash-free, 18.77 per cent. N. All these analyses are here brought together.

HEMP-SEED GLOBULIN.

	Ritthausen.				Osborne.	
	d.	*e.*	*f.* Crystals.	*g.*	**4.** Crystals.	**5.** Crystals.
Carbon	50.94	51.33	50.98	51.19	51.26	51.28
Hydrogen	6.85	6.84	6.92	6.97	6.86	6.84
Nitrogen	18.62	18.80	18.73	18.06	18.68	18.84
Sulphur	0.85	0.82	0.82	0.92	0.94	0.87
Oxygen	22.74	22.21	22.55	22.86	22.26	22.17
	100.00	100.00	100.00	100.00	100 00	100.00

The crystallised preparations, *f*, **4** and **5** are in close agreement in all respects, excepting the carbon of *f*, which is somewhat low. Ritthausen's analyses *e* and *g* agree with the writer's and it is therefore almost certain that these are very nearly correct. That Ritthausen's carbon was a little too low was probably due to the fact that these crystalline preparations absorb moisture with great rapidity. Preparations which had been dried at 110° and preserved in closely-corked bottles in a desiccator were found to have absorbed from 0.5 to 1.0 per cent. of moisture in consequence

of the brief exposure to the air incurred in removing the portions required for nitrogen determinations. The figures obtained for nitrogen agree as closely as could be expected, with the exception of that for *g*, which is unquestionably too low.

As **5** was perfectly crystallised and undoubtedly pure, its analysis may safely be taken as closely representing the true composition of this proteid. The reactions of this body were found to be as follows:

In pure water **4** was wholly insoluble, even on heating to 50°; on the other hand, **5** yielded a turbid solution with distilled water, becoming very nearly clear on warming to 50°. **4** was prepared by dialysis; **5** by cooling a warm sodium chloride solution. A similar difference for preparations made in these two ways was noticed in the globulin of the oat-kernel,[1] and also in that of the castor-bean, next to be described. The solution in water is precipitated on adding a little sodium chloride; the precipitate so produced being soluble on a further addition of this salt. **4** is wholly insoluble in glycerin, either concentrated or dilute, while **5** dissolves to a perfectly clear liquid, which is not precipitated by dilution, but is precipitated by adding a little salt, the substance redissolving on adding more salt. Both preparations dissolve in solutions of sodium chloride at 20°. The solubility increases with the temperature up to 60° or over. Near 0° very little, if any, dissolves in 10-per cent. sodium chloride solution. Saturation of the sodium chloride solution of this globulin with salt produces a trifling precipitate; saturation with ammonium sulphate gives a complete precipitation, as does also saturation with magnesium sulphate. It is very soluble in distilled water containing a minute amount of acid. Preparation **5** dissolves instantly and completely in $\frac{5}{1000}$-per cent. hydrochloric acid, provided enough is taken to combine with all the substance present. **4**, on the other hand, requires a somewhat stronger acid, but is readily soluble in $\frac{2}{100}$-per cent. hydrochloric acid. Very dilute solutions of sodium carbonate (about $\frac{1}{10}$ per cent.) dissolve both preparations readily and completely, as do also solutions of caustic alkalies. Dissolved in 10-per cent. sodium chloride solution, a turbidity occurs on heating to 75°, and flocks form at 86°. If the solution is then filtered and again heated, turbidity results a second time at 88°, flocks forming at 95°. The amount of substance separated

[1] This Journal **13**, 408.

at 86° is hardly more than a trace and undoubtedly indicates the presence of an extremely small amount of some other proteid, for if the sodium chloride solution is saturated with salt and the trifling precipitate produced is filtered off and the filtrate diluted until it contains 10-per cent. of sodium chloride, it first becomes turbid when heated to 87°, and no flocks appear until heated to 94°. If the solution is then raised to 98°, kept at that temperature for some time, filtered from the coagulum and boiled, no more precipitate is produced. If acid be added a very heavy precipitate falls, showing either that the globulin is only partly precipitated at 100°, or that it undergoes a change, a portion being converted into a proteid unaffected by boiling. This substance gives all the reactions common to proteids.

The crystals in which this proteid usually separates are simple octahedrons, although modifications of this form are frequently to be seen. Occasionally hexagonal plates may be found among the octahedrons. In two cases where this body was crystallised in small quantity, the entire crop of crystals consisted of very thin hexagonal plates.

The facility with which crystals of this proteid can be obtained is very remarkable, it separating in this form whenever precipitated by dialysing or by cooling its solutions. The other seeds from which crystallised proteids have thus far been obtained yield well-crystallised products only with considerable difficulty, as the proteid usually separates in the spheroidal form.

Crystalline Proteid of the Castor-bean.

Ritthausen[1] first obtained the globulin of the castor-bean in a well-crystallised condition by following Grübler's method. Subsequently[2] he gave the results of his analyses of this substance (*h*), which were as follows:

As the mean of several analyses of two preparations, previously described,[3] obtained by extracting the seeds with sodium chloride solution and precipitating the extracts by diluting and passing through them carbonic acid gas, Ritthausen also gave the figures *i* and *j*.

[1] J. prakt. Chem. **23**, 481. [2] *Ibid.* **25**, 130.
[3] Pflüger's Archiv **19**, 15.

CASTOR-BEAN GLOBULIN.—*Ritthausen.*

	h. Crystals.	*i.*	*j.*
Carbon	50.88	52.05	51.31
Hydrogen	6.98	6.83	6.90
Nitrogen	18.58	18.57	18.43
Sulphur	0.77	0.96	0.97
Oxygen	22.79	21.59	22.39
	100.00	100.00	100.00

As these analyses leave the composition of this proteid in doubt, and since many of the reactions now considered important were not given by Ritthausen, this substance has been reinvestigated by the writer. The material for the preparation of this proteid was obtained by freeing the castor-beans from their shells, bruising the meats in a mortar, extracting the oil with ether, and pulverising the residue. 60 grams of this meal were extracted with 10-per cent. sodium chloride brine and the filtered extract placed in a dialyser. After removal of the chlorides, the separated proteid was filtered off, washed with water, alcohol and ether, and dried over sulphuric acid. 4.25 grams of material were so obtained, consisting of spheroids and crystals mixed with intermediate forms. After drying at 110°, this preparation was analysed with the following result:

CASTOR-BEAN GLOBULIN: *Crystals and Spheroids.—Preparation* 6.

		Ash-free.
Carbon	51.57	51.62
Hydrogen	6.92	6.92
Nitrogen	18.79	18.81
Sulphur	0.76	0.76
Oxygen	..	21.89
Ash	0.10	..
		100.00

Ash.—0.5615 gram substance, dried at 110°, gave 0.0006 gram ash = 0.10 per cent.

Carbon and hydrogen.—0.3069 gram dried substance gave 0.5803 gram CO_2 = 51.57 per cent. C, and 0.1914 gram H_2O = 6.92 per cent. H.

Nitrogen.—0.2855 gram dried substance gave 43.64 cc. N at 6° (barometer 763.3 mm. at 23°) = 18.79 per cent.

Sulphur.—0.9173 gram dried substance gave 0.0503 gram $BaSO_4$ = 0.76 per cent. S.

Another extraction was made by treating 75 grams of the fine oil-free meal with 1000 cc. of a 5-per cent. sodium chloride solution heated to 60°, filtering perfectly clear and allowing the extract to cool to 5°. A considerable amount of substance separated, which was filtered off and washed with water, in which it was found to dissolve when the salt was nearly all removed. The entire precipitate was then dissolved in 10-per cent. sodium chloride brine and the resulting clear solution saturated with salt. This produced a considerable precipitate, which was filtered off and washed with a saturated salt solution. The filtrate and washings were then put in a dialyser.

The portion precipitated by saturation with sodium chloride was dissolved in a dilute solution of this salt and likewise submitted to dialysis. After the contents of the dialysers had become free from chlorides the precipitates were filtered off, washed with alcohol and ether, and dried over sulphuric acid. The preparation obtained from the *precipitate* produced by saturation with sodium chloride weighed 3.3 grams and consisted of imperfectly formed crystals and spheroids; while that obtained from the saturated salt *solution* weighed 2.5 grams and was likewise partly crystalline. These two preparations were analysed, the composition of the former being shown by the analysis of preparation **7**, the latter by that of **8**.

CASTOR-BEAN GLOBULIN: *Crystals and spheroids insoluble in saturated sodium chloride solution*—**7**.

	Ash-free.		Average.
	I.	II.	
Carbon	51.40	51.29	51.35
Hydrogen	6.81	6.86	6.84
Nitrogen	18.88	..	18.88
Sulphur	0.79	..	0.79
Oxygen	..	..	22.14
Ash	0.00	..	..
			100.00

Ash.—0.6773 gram substance, dried at 110°, gave no weighable ash.

Carbon and hydrogen, I.—0.3614 gram dried substance gave 0.6811 gram CO_2 = 51.40 per cent. C, and 0.2215 gram H_2O = 6.81 per cent. H.

II.—0.3544 gram dried substance gave 0.6664 gram CO_2 = 51.29 per cent. C, and 0.2187 gram H_2O = 6.86 per cent. H.

Nitrogen.—0.3481 gram dried substance gave 53.55 cc. N at 6° (barometer 762.3 mm. at 23.5°) = 18.88 per cent.

Sulphur.—0.7960 gram dried substance gave 0.0460 gram $BaSO_4$ = 0.79 per cent. S.

CASTOR-BEAN GLOBULIN: *Crystals and spheroids soluble in saturated sodium chloride solution*—8.

	I.	II.	Average.	Ash-free.
Carbon	51.14	50.99	51.07	51.19
Hydrogen	6.82	6.81	6.82	6.84
Nitrogen	18.88	..	18.88	18.92
Sulphur } Oxygen }	..	..	..	23.05
Ash	0.24	..	0.24	..
				100.00

Ash.—0.4480 gram substance, dried at 110°, gave 0.0011 gram ash = 0.24 per cent.

Carbon and hydrogen, I.—0.3483 gram dried substance gave 0.6531 gram CO_2 = 51.14 per cent. C, and 0.2137 gram H_2O = 6.82 per cent. H.

II.—0.3485 gram dried substance gave 0.6516 gram CO_2 = 50.99 per cent. C, and 0.2136 gram H_2O = 6.81 per cent. H.

Nitrogen.—0.2987 gram dried substance gave 46.05 cc. N at 6° baro meter 760.2 mm. at 22°) = 18.88 per cent.

The filtrate from the precipitate, produced by cooling the 5-per cent. sodium chloride extract last described, was heated to 40° in a water-bath, and twice its volume of distilled water heated to 40° was added. A perfectly clear solution resulted, which when slowly cooled to 5° yielded a well-crystallised precipitate, the crystals being almost exclusively octahedrons with one angle truncated. This precipitate was filtered off, washed with water, alcohol and ether, and when dried over sulphuric acid weighed 3.5 grams. Dried at 110°, this preparation was found to have the following composition:

CASTOR-BEAN GLOBULIN: *Crystals—Preparation* 9.

	I.	II.	Average.	Ash-free.
Carbon	51.31	51.26	51.29	51.31
Hydrogen	6.94	7.00	6.97	6.97
Nitrogen	18.75	..	18.75	18.75
Sulphur	0.70	0.82	0.76	0.76
Oxygen	..	..	..	22.21
Ash	0.03	..	0.03	..
				100.00

Ash.—0.6315 gram substance, dried at 110°, gave 0.0002 gram ash = 0.03 per cent.

Carbon and hydrogen, I.—0.3157 gram dried substance gave 0.5939 gram CO_2 = 51.31 per cent. C, and 0.1969 gram H_2O = 6.94 per cent. H.

II.—0.3976 gram dried substance gave 0.7473 gram CO_2 = 51.26 per cent. C, and 0.2516 gram H_2O = 7.00 per cent. H.

Nitrogen.—0.3074 gram dried substance gave 48.1 cc. N at 6° (barometer 744.0 mm. at 21°) = 18.75 per cent.

Sulphur, I.—0.5468 gram dried substance gave 0.0273 gram $BaSO_4$ = 0.70 per cent. S.

II.—0.4588 gram dried substance gave 0.0275 gram $BaSO_4$ = 0.82 per cent. S.

The following table shows the results of the analyses of Ritthausen and the writer:

CASTOR-BEAN GLOBULIN.

	Ritthausen.			Osborne.			
	h Crystals.	*i*	*j*	6 Crystals and Spheroids.	7	8	9 Crystals.
Carbon	50.88	52.05	51.31	51.62	51.35	51.19	51.31
Hydrogen	6.98	6.83	6.90	6.92	6.84	6.84	6.97
Nitrogen	18.58	18.57	18.43	18.81	18.88	18.92	18.75
Sulphur	0 77	0.96	0.97	0.76	0.79	23.05	0.76
Oxygen	22.79	21.59	22.39	21.89	22.14		22.21
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

This table shows plainly that Ritthausen's figure for carbon in the analysis of his crystallised preparation is too low, probably because the sample had taken up a little moisture from the air, as explained before in discussing the analyses of the hemp-seed proteids. Apart from this, Ritthausen's analysis of his crystallised preparation agrees well with those of the writer.

The analysis of the perfectly crystallised preparation **9** may be taken as closely representing the true composition of this proteid.

In pure water this substance behaved like that obtained from the hemp-seed—that is, preparations separated by dialysis were insoluble in water, either at 20° or 50°; those separated by cooling warm saline solutions were soluble to opalescent solutions at 20°, becoming clear on warming. Solutions in water are precipitated by a small quantity of sodium chloride, the precipitate dissolving on adding a larger quantity of the salt. Preparations **7** and **8**, which dissolved in water when first separated from the warm

solution, after being redissolved and then precipitated by dialysis, were wholly insoluble in water.

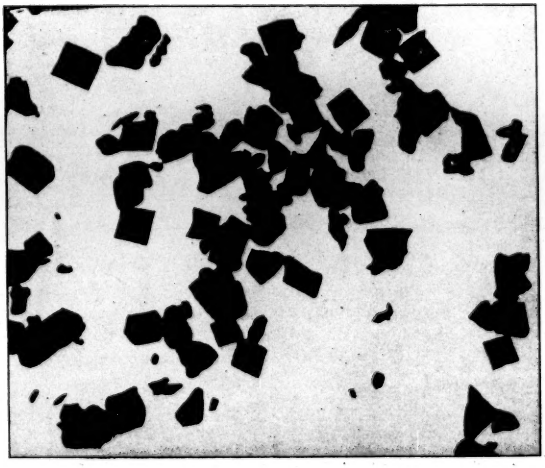
Toward glycerin this proteid behaves in the same way as the hemp-seed globulin. Some of the preparations made dissolved completely, others partly, in a 10-per cent. sodium chloride solution, leaving a considerable insoluble residue (Weyl's "albuminate"), which, however, dissolved almost wholly on warming to 50°, a part separating in spheroids when the solution was cooled.

Saturation of the salt-solution with ammonium sulphate completely precipitates this proteid. Saturation with sodium chloride precipitates a large part of the globulin contained in the extract of the seed, the part precipitated and that remaining in solution being alike in most of their reactions and composition.

It is probable that the precipitate produced by saturation with sodium chloride solution is caused by a small amount of some other substance extracted from the seed, for the crystalline preparation **9**, which was obtained from the extract after previous precipitation of a part of the globulin contained in it, gave on saturation with this salt only a trifling precipitate. A part of the precipitate is also unquestionably due to a small amount of another globulin coagulating at from 75° to 86°, for where this precipitate is dissolved in 10-per cent. sodium chloride brine and heated to this temperature, a much larger coagulum is produced than when the substance obtained from the salt-saturated solution is similarly treated. The perfectly crystalline preparation **9**, which yields but a very small amount of precipitate on saturating its salt-solution with sodium chloride, likewise gives but a trace of coagulum when heated to 89°. The crystalline globulin is therefore not precipitated by saturation with sodium chloride.

Dissolved in 10 per cent. sodium chloride solution, the crystalline preparation **9** became turbid when heated to 87°, and at 89° a very minute amount of flocks separated. At 95° a large coagulum developed and, on heating the solution for some time at 98°, after filtering off the coagulum, the filtrate gave only a turbidity when boiled, but on adding acid a very large precipitate fell, showing that the globulin is but partly precipitated by heat.

The other preparations behaved, on heating their salt-solutions, in exactly the same way, except that the coagulum separating at the lower temperature was somewhat greater in amount.



CRYSTALLISED GLOBULIN OF FLAX-SEED.

This proteid can be crystallised only with difficulty, being in this respect very different from the globulin of the hemp-seed. It is not improbable that this is due to other constituents of the seed which interfere to a greater or less extent with the formation of the crystals.

The Crystalline Proteid of the Flax-seed.

In a former paper[1] the writer has described a crystallised globulin obtained from flax-seed, the appearance of the crystals being shown by the accompanying plate. As the mean of a large number of accordant analyses its composition was given as follows:

Crystallised Flax-seed Globulin.

Carbon	51.48
Hydrogen	6.94
Nitrogen	18.60
Sulphur	0.81
Oxygen	22.17
	100.00

In pure distilled water at 20° this proteid, whether separated from solution by cooling or by dialysis, is wholly insoluble, while in water at 40° it is very slightly soluble. In 10 per cent. sodium chloride solution it is mostly soluble, a part (Weyl's "albuminate") generally remaining undissolved, which, however, dissolves readily on warming the solution and partly separates, on cooling, in very finely developed crystals. In glycerin diluted with water the substance separated by dialysis is wholly insoluble, either at 20° or 40°; while the substance separated from a warm sodium chloride solution is largely soluble at 20°.

Saturation with ammonium sulphate and also with magnesium sulphate completely precipitates this proteid from its solutions. Saturation with sodium chloride gives a small precipitate which consists partly of other globulins, for the filtrate from this precipitate, when diluted so as to contain 10 per cent. of sodium chloride, yields but a trace of coagulum on heating to boiling, while the precipitate dissolved in 10-per cent. sodium chloride coagulated at the various temperatures observed in the solution before saturation.

[1] This Journal **14**, 629.

In very dilute acids—as $\frac{1}{5}$-per cent. hydrochloric acid or $\frac{1}{10}$-per cent. citric acid—the proteid readily and completely dissolves to solutions which are precipitated by adding a little sodium chloride. In $\frac{1}{10}$-per cent. sodium carbonate solution or $\frac{1}{100}$-per cent. potash solution the substance dissolves at once.

On heating a 10-per cent. sodium chloride solution of this globulin small amounts of substance separate at different temperatures, according to the method employed in the preparation of the proteid. The substances coagulating below 90° are undoubtedly traces of other globulins, as already stated. These globulins are also separated from the crystalline globulin if the latter is precipitated from solution by cooling, and after washing with water, alcohol and ether and drying over sulphuric acid, is then dissolved in 10-per cent. sodium chloride brine. Thus separated from these coagulable proteids, boiling produces but a trace of a coagulum, while in the case of the globulin obtained by dialysis the coagulum produced by boiling is a little greater. In any case the coagulation produced by boiling is only partial, for a very large precipitate is always obtained on adding acid to these solutions, even after boiling them for some time. The crystals of this proteid were always obtained as octahedrons.

The Crystallised Proteid of the Oat-kernel.

In this Journal[1] and also in the Report of the Connecticut Agricultural Experiment Station for 1891, the writer described a globulin from the oat-kernel which was obtained in a perfectly crystallised condition. The form of these crystals is shown in the plate.

As an average of three closely agreeing analyses of different preparations, its composition was found to be as follows:

Crystallised Oat-globulin.

Carbon	52.18
Hydrogen	7.05
Nitrogen	17.90
Sulphur	0.53
Oxygen	22.34
	100.00

[1] This Journal **13**, 408; **14**, 212.

CRYSTALLISED GLOBULIN OF THE OAT KERNEL.

In distilled water at 20° this proteid in the crystallised condition is not soluble; in the spheroidal condition, separated from a warm sodium chloride solution, by boiling it yields an opalescent solution. On slowly warming, the crystals partly dissolve, while the spheroids quickly give a clear solution. Addition of a little sodium chloride precipitates these solutions, but the addition of a further quantity of this salt redissolves the precipitate. In 10-per cent. sodium chloride the crystals dissolve at 20°, with the exception of a slight residue which is nearly completely dissolved by warming. This solution is not precipitated by boiling. Dilution precipitates the proteid.

In diluted glycerin most of the substance dissolves. Saturation with ammonium sulphate and with magnesium sulphate completely precipitates solutions of this proteid in sodium chloride brine. Saturation with sodium chloride likewise precipitates such solutions. In very dilute acids, $\frac{2}{100}$-per cent. hydrochloric acid or $\frac{1}{2}$-per cent. citric acid, it is readily soluble to a perfectly clear solution. In $\frac{1}{2}$-per cent. sodium carbonate solution the crystals are soluble with the exception of a slight residue; the spheroids, on the other hand, with $\frac{1}{10}$-per cent. sodium carbonate give at once a perfectly clear solution. In $\frac{1}{100}$-per cent. potash solution both spheroids and crystals are at once dissolved to a perfectly clear solution.

The Crystallised Proteid of the Squash-seed.

This proteid was first prepared and analysed by Barbieri,[1] whose preparations, however, were not crystalline; soon after Grübler,[2] employing a method suggested by Drechsel, succeeded in obtaining perfectly crystallised preparations of this substance and published the results of his observations and analyses. The percentage of carbon found by Grübler was shown by Ritthausen[3] to be too high, owing to the use of an imperfect method for the determination of this element.

Later Chittenden and Hartwell[4] prepared this proteid in the crystallised form for use in digestion experiments, and published analyses confirming those of Ritthausen. Grübler[5] has lately made a new analysis of this body nearly according with the results of Ritthausen and with those of Chittenden and Hartwell.

[1] J. prakt. Chem. [2] **18**, 102. [2] *Ibid.* [2] **23**, 97. [3] *Ibid.* [2] **23**, 481.
[4] J. Physiol. **11**, 435. [5] J. prakt. Chem. [2] **44**, 369.

As, however, the analyses of these investigators do not harmonise in all respects, the writer has examined this proteid with the hope of fixing its composition more nearly.

A quantity of squash-seeds, freed from hulls and finely chopped, were extracted with ether. The coarse powder was then thrown on a straining cloth and the finest portions washed through together with the ether. The coarse meal remaining in the cloth was then ground in a mortar and again washed on the cloth with ether. After freeing from most of the oil and drying in the air, 50 grams of coarse meal were obtained from 200 grams of freshly-shelled meats. The fine meal passing through the cloth was collected on a filter and washed with ether until fat-free, and, after drying in the air, weighed 70 grams, all of which passed through a sieve with holes $\frac{1}{50}$-inch in diameter.

Fifty grams of the coarse meal were then treated with 800 cc. of 2-per cent. sodium chloride solution heated to 60°, and at once thrown on to a filter. The clear extract was allowed to cool in a beaker left over night in a room having a temperature of about 10°. In the morning a large deposit had separated, entirely composed of perfectly formed octahedrons. This was filtered off, washed once with water and then thoroughly with 50-per cent. alcohol, then with absolute alcohol and finally with ether, and dried over sulphuric acid. A snow-white preparation, weighing 9 grams, was thus obtained, which was dried at 110° and analysed with the following results:

SQUASH-SEED PROTEID: *Crystallised—Preparation* **10.**

	I.	II.	III.	Average.	Ash-free.
Carbon	51.49	51.64	51.74	51.62	51.66
Hydrogen	6.88	6.92	6.84	6.88	6.89
Nitrogen	18.50	18.49	...	18.50	18.51
Sulphur	0.88	0.87	...	0.88	0.88
Oxygen	...	...	...	...	22.06
Ash	0.08	...	...	...	...
					100.00

Ash.—0.4789 gram substance, dried at 110°, gave 0.0004 gram ash = 0.08 per cent.

Carbon and hydrogen, I.—0.3868 gram dried substance gave 0.7303 gram CO_2 = 51.49 per cent. C, and 0.2394 gram H_2O = 6.88 per cent. H.

II.—0.3986 gram dried substance gave 0.7548 gram CO_2 = 51.64 per cent. C, and 0.2484 gram H_2O = 6.92 per cent. H.

III.—0.3116 gram dried substance gave 0.5917 gram $CO_2 = 51.74$ per cent. C, and 0.1919 gram $H_2O = 6.84$ per cent. H.

Nitrogen, I.—0.2577 gram dried substance gave 39.21 cc. N at 10° (barometer 765.4 mm. at 21°) = 18.50 per cent.

II.—0.3881 gram dried substance gave 59.32 cc. N at 11° (barometer 764.7 mm. at 24°) = 18.49 per cent.

Sulphur, I.—0.7086 gram dried substance gave 0.0457 gram $BaSO_4 = 0.88$ per cent. S.

II.—0.7804 gram dried substance gave 0.0493 gram $BaSO_4 = 0.87$ per cent. S.

Another preparation was made by extracting 50 grams of the fine meal with 10-per cent. sodium chloride solution at 20°, filtering from the insoluble residue and dialysing the clear extract. As the chloride was removed the proteid precipitated in the form of irregular granules together with some spheroids and crystals. After dialysing three days the large deposit was filtered off, washed with water, alcohol and ether, and dried over sulphuric acid. This preparation weighed 21.27 grams, and when dried at 110° was analysed with the following result:

SQUASH-SEED PROTEID: *Spheroids—Preparation* II.

	I.	II.	Average.	Ash-free.
Carbon	51.31	51.23	51.27	51.42
Hydrogen	6.82	6.80	6.81	6.83
Nitrogen	18.50	18.68	18.59	18.64
Sulphur	0.90	0.89	0.90	0.90
Oxygen	...	...	...	22.21
Ash	0.29	...	...	...
				100.00

Ash.—1.1487 grams substance, dried at 110°, gave 0.0033 gram ash = 0.29 per cent.

Carbon and hydrogen, I.—0.3835 gram dried substance gave 0.7215 gram $CO_2 = 51.31$ per cent. C, and 0.2356 gram $H_2O = 6.82$ per cent. H.

II.—0.3743 gram dried substance gave 0.7032 gram $CO_2 = 51.23$ per cent. C, and 0.2289 gram $H_2O = 6.80$ per cent. H.

Nitrogen, I.—0.3313 gram dried substance gave 50.64 cc. N at 11° (barometer 765 3 mm. at 23°) = 18.50 per cent.

II.—0.3761 gram dried substance gave 58.13 cc. N at 11° (barometer 764.0 mm. at 24°) = 18.68 per cent.

Sulphur, I.—0.6426 gram dried substance gave 0.0424 gram $BaSO_4 = 0.90$ per cent. S.

II.—0.8344 gram dried substance gave 0.0545 gram $BaSO_4 = 0.89$ per cent. S.

The following table gives the results obtained by all the chemists who have analysed this substance:

		Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.
Barbieri, amorphous		51.88	7.51	18.08	0.60	21.93
Grübler's 1st analyses,	crystals	53.21	7.22	19.22	1.07	19.10
Ritthausen	"	51.61	7.00	...	...	...
Chittenden and Hartwell,	"	51.60	6.97	18.80	1.01	21.62
" "	spheroids	52.03	6.93	19.08	1.04	20.92
" "	amorphous	51.81	6.94	18.71	1.01	21.53
Grübler's 2d analyses,	crystals	51.48	6.76	18.14	0.96	21.53
10 Osborne	"	51.66	6.89	18.51	0.88	22.06
11 "	spheroids	51.42	6.83	18.64	0.90	22.21

If we omit Grübler's first analysis, which, as already stated, is incorrect, the average of the remaining eight is

Carbon	51.69
Hydrogen	6.97
Nitrogen	18.57
Sulphur	0.91
Oxygen	21.86
	100.00

The reactions of the preparations made by the writer were as follows:

In distilled water either at 20° or warmed to 60° the preparations, whether separated from solution in sodium chloride brine by dialysis or by cooling, were wholly insoluble. In glycerin, either strong or diluted, they were likewise insoluble, even when warmed to 60°. In 10-per cent. sodium chloride solution they both dissolved with the exception of a mere trace, and the resulting solutions were not precipitated by saturating with sodium chloride, but were completely precipitated by saturation with ammonium sulphate and also with magnesium sulphate. In $\frac{2}{100}$-per cent. hydrochloric acid and $\frac{1}{10}$-per cent. citric acid both yielded perfectly clear solutions when sufficient acid was employed to form an acid compound with the proteid. In $\frac{1}{10}$-per cent. sodium carbonate and $\frac{1}{100}$-per cent. potash solutions the preparations dissolved readily and completely; these solutions were not precipitated by sodium chloride. When dissolved in 10-per cent. sodium chloride solution and heated to coagulation the two preparations behaved somewhat differently, as is best shown by the following table: A being the preparation obtained by cooling the

warm 2-per cent. sodium-chloride extract; B, that precipitated by dialysis.

HEAT-COAGULATION OF SQUASH-SEED GLOBULIN.

	A.	B.
Turbidity at	..	73° } Small
Flocks at	..	84 } precipitate.
After filtering at	87°	..
Turbidity	87	87 [?]
Flocks	95	95
After filtering and boiling	no coagulum.	no coagulum.

On adding a drop of acetic acid both yielded a large precipitate. These figures indicate that B contains a small amount of some other globulin which coagulates between 73° and 84°.

CONCLUSIONS.

I. The data presented in this paper indicate that the crystallised globulins of the Brazil-nut and of the oat-kernel are distinct substances. To facilitate comparison, analyses of these two globulins are here stated as follows:

	BRAZIL-NUT. I.	OAT-KERNEL. Average.
Carbon	52.18	52.18
Hydrogen	6.92	7.05
Nitrogen	18.30	17.99
Sulphur	1.06	0.53
Oxygen	21.54	22.34
	100.00	100.00

If the differences in nitrogen- and sulphur-content are not perhaps sufficient to distinguish these two proteids, their reactions prove them to be distinct, for when prepared in the same manner they are unlike in many respects.

In distilled water heated to 60° the globulin of the Brazil-nut is wholly insoluble, while that of the oat-kernel dissolves completely. Saturation of a 10-per cent. sodium chloride solution of these substances with salt almost completely precipitates the proteid of the oat-kernel, that of the Brazil-nut being unaffected. Saturation of similar solutions with magnesium sulphate precipitates but little of the Brazil-nut, but all of the oat-globulin. When solutions of these bodies in 10-per cent. sodium chloride brine are heated, the Brazil-nut globulin begins to separate at 70°, a flocculent coagulum forming at 84°, which increases on raising the temperature to boiling, the proteid being largely, but not

wholly, precipitated. The globulin of the oat-kernel, on the other hand, is not coagulated at all by boiling.

II. The crystalline globulins of the hemp-seed, castor-bean, squash-seed and flax-seed are almost identical in composition, as may be seen by comparing the analyses:

	Hemp-seed. 5.	Castor-bean. 9.	Squash-seed. 10.	Flax-seed. Average.
Carbon	51.28	51.31	51.66	51.48
Hydrogen	6.84	6.97	6.89	6.94
Nitrogen	18.84	18.75	18.51	18.60
Sulphur	0.87	0.76	0.88	0.81
Oxygen	22.17	22.21	22.06	22.17
	100.00	100.00	100.00	100.00

The carbon content of the hemp- and castor-globulins is less than that of the squash- and flax-globulins by about 0.25 per cent., a difference too slight to have importance, if it were not constant for almost all the preparations analysed. The deportment of these globulins toward reagents is also very similar, but in this respect the hemp- and castor-globulins show slight differences from those of the squash- and flax-seed; the two former are, however, almost exactly alike, and the two latter likewise closely agree together. The following table plainly exhibits these relations:

HEAT-COAGULATIONS.

	Hemp.	Castor.	Squash.	Flax.
Turbidity,	75°	87°	..	67°
Flocks,	86	89	..	80
Filtered,				
Turbidity,	88	87	87	90
Flocks,	95	95	95	96
Filtered,				
Boiling,	no coagulum.	ditto.	ditto.	ditto.
Filtered and acetic acid added,	heavy pp.	ditto.	ditto.	ditto.

SOLUBILITY.[1]

	Hemp.[2]		Castor.		Squash.		Flax.	
	A.	B.	A.	B.	A.	B.	A.	B.
H_2O at 20°,	P.	I.	P.	I.	I.		I.	I.
H_2O at 40°,	S.	I.	S.	I.	I.		P.	I.
Glycerine at 20°,	S.	I.	S.	I.	I.		P.	I.
Glycerine at 50°,	S.	I.	S.	I.	I.		P.	I.
10-per cent. NaCl sol. diluted,	Pp.	Pp.	Pp.	Pp.	Pp.		Pp.	Pp.
Saturated with NaCl,	T. pp.	T. pp.	T. pp.	T. pp.	No Pp.		V.	V.
Saturated with $MgSO_4$,	C. pp.	C. pp.	C. pp.	C. pp.	C. pp.		C. pp.	C. pp.

[1] P. = Partly soluble; I. = Insoluble; S. = Soluble; Pp. = Precipitate; T. pp. = Trace of precipitate; C. pp. = Completely precipitated; V. = Very little precipitate.

[2] A indicates preparations obtained by cooling warm salt solutions; B, those obtained by dialysis.

As already stated, the proteids coagulating at the lower temperature are traces of other globulins imperfectly separated from the crystalline globulin. The coagulum separating at the higher temperature is undoubtedly a part of the crystalline globulin which is broken up when heated to this temperature. It is seen that the temperature at which this coagulum separates is the same for all four substances.

In solubility these four proteids are very nearly alike, the most noticeable difference being that the globulins of the hemp-seed and castor-bean, when separated from a warm sodium-chloride solution, are soluble in water and diluted glycerin, while the other preparations, both of this substance and the globulins of other seeds, are insoluble under the same conditions.

Of the flax-seed globulin separated from a warm salt solution, a little dissolves in water at 40°.

The small precipitate obtained by saturating a sodium-chloride solution of the globulins with salt undoubtedly consists mostly of traces of other globulins.

It is at present impossible to assert that these four globulins are the same, but since differences exist between different preparations of globulin from the same seed as great as those found among the globulins of these different seeds, the writer is disposed to consider these four globulins as identical.

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